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NUTRITIONAL SURVEY IN NORRIS POINT, NEWFOUNDLAND

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INTRODUCTION

NUTRITIONAL studies have suggested that malnutrition is an extensive and important problem in Newfoundland. Little[1, 2] described beriberi in Newfoundland, stressing in particular its occurrence in association with a diet restricted largely to white flour. Appleton,[3] Aykroyd,[4, 5] Mitchell,[6] Vaughn and Mitchell,[7] and Steven and Wald[8] studied dietaries and deficiency diseases in Newfoundland and Labrador. A high incidence of beriberi, vitamin A deficiency, dental caries, spongy hypertrophied gums, and chronic malnutrition was noted. Olds[9] and McDevitt and co-workers,[10] working in widely separated areas of Newfoundland, found low levels of ascorbic acid in the blood in a high percentage of persons tested. The most extensive survey of nutrition in Newfoundland is the recent report by Adamson and associates.[11] These workers examined 868 people and did certain chemical analyses on the body fluids of nearly half of the subjects seen. Deficiencies of vitamin A, riboflavin, and ascorbic acid were frequent.

The present report describes a nutritional survey at Norris Point, during June, 1944, made with a twofold purpose: (1) rapid assessment of nutritional status by examination of a weighted population sample; (2) establishment of a baseline from which the future nutrition of the same or similar samples can be judged. It was particularly desirable to establish this baseline, for in the near future enriched flour was to be introduced into Newfoundland.‖

Norris Point is a west coast fishing and logging village of approximately 100 families. It is typical of many such villages, the inhabitants of which make up a large part of the total population of Newfoundland. Many factors favor the existence of malnutrition: (1) geographic isolation; (2) primitive transportation; (3) minimal agricultural production on leached marginal land by obsolete wasteful methods; (4) inadequate educational facilities; and (5) poor housing, food storage, and cooking facilities.

The Scotch-English stock at Norris Point, which comprised the sample, had all lived under such influences for at least two years, many for generations. The incidence of blood relationship was high. Certain colloquialisms of speech, together with reticence and shyness, particularly in the presence of strangers, made accurate history-taking difficult.

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‖By order of the Newfoundland Government and on recommendation of the Council on Nutrition of the Newfoundland Medical Association, all flour entering the country after July 1, 1944, is to be enriched to new American standards.

MATERIAL AND METHODS

The population sample was selected from groups known to be particularly susceptible in regard to clinical deficiency disease, notably pregnant and lactating women and rapidly growing children. Such groups may be considered as a sample so weighted as to uncover nutritional deficiency if any exists. This sample is not representative of the general nutrition of the community but rather is likely to approximate the poorest nutrition. It was felt that if these groups showed no evidence of malnutrition, no great amount of malnutrition would be present in the population at large.

The following criteria were established. The family was made the unit of study, and only those families in which there had been a pregnancy within the previous two years were included. In addition to the women of such families, their children between the ages of 3 and 6 and 10 and 14 years were studied, while other children were excluded. Husbands in such families were examined when available but often were absent in fishing or lumbering industries. The total group was homogeneous with regard to socioeconomic status. Of the 40 families in Norris Point which fulfilled these criteria, all but one agreed to take part in the survey. The sample of thirty-nine families had the following composition: women, 39; children 3-6 years of age, 34 (21 boys, 13 girls); children 10-14 years of age, 24 (10 boys, 14 girls); men, 6. Of the women examined, 6 were pregnant and 14 lactating. Ten additional men seen in the clinic were included in the survey.

The 113 individuals were examined by a survey team of 6 members in a period of ten days. The local physician administered the project and acted as liaison officer in the selection of the sample. Three physicians obtained medical histories and conducted the physical examinations. All positive physical findings were observed by all three physicians and recorded only if there was a majority agreement. Two research associates obtained nutritional histories and were responsible for photography and assistance in laboratory work.

The complete examination consisted of five parts: (1) A nutritional history was taken, consisting of a record of all nourishment ingested in the previous twenty-four hours. A complete list of all available foods was used to remind the individuals of foods they may have eaten and to uncover any peculiarities in the diet on the day of the test. At the same time the frequency of intake of protective foods per day and week was recorded. (2) A detailed medical history was taken, and this was later supplemented by the local hospital records. (3) A thorough and extensive physical examination was made with special attention given to eyes, mouth, skin, and neurological findings. Height and weight were recorded on all individuals, and in the case of children the intercristal diameter was measured. (4) Photographs consisted of two types: (a) color films of all lesions; (b) nude full-length black and white photographs taken of most children at a fixed distance, against a standard measuring scale. (5) Laboratory tests undertaken included the usual routine urinalysis for qualitative detection of sugar, albumin, and cellular material, and the determination of hemoglobin and total plasma protein from a 2 to 3 ml. sample of venous blood. The gravimetric copper sulfate method of Phillips and associates[12] was used. The recording was so planned that all necessary data could conveniently be checked off or indicated on 5 by 8 filing cards. Diagrams of body surface, face, and tongue were included on these cards and proved to be particularly useful in indicating the exact location and extent of lesions and in recalling the various cases later when the data were assembled.

FINDINGS

Nutritional History.—The diets of all of the families examined showed remarkable similarity. Children, as soon as they are weaned, receive the same food as adults. A typical daily menu is given in Table I. The main protein foods are fish (largely salt cod and herring in the winter), salt pork, beef (with much fat), dried beans, and peas. Occasionally some fresh meat is consumed when wild game can be obtained or when a sheep, cow, or pig is slaughtered. Lack of storage facilities makes the supply of fresh meat largely dependent on the weather. The supply of fresh fish (cod, salmon, lobster, herring, tuna, trout, caplin) is dependent on the run in a particular year. Margarine, fortified with 6,000 I.U. of vitamin A per pound, is used as practically no butter is available. Fresh vegetables are not available from October to July, and during this period root vegetables, including potatoes, turnips, carrots, parsnips, and beets, are used in addition to cabbage, which may be either fresh or salted. Potatoes are eaten about twice daily unless the family supply is exhausted before the winter ends. A few families eat green leafy vegetables in the summer months.

TABLE I. TYPICAL DAILY MENU

Breakfast	*Dinner*	*Supper*
Porridge with milk (30 to 40 ml.) and sugar or molasses	Fish (salt or fresh), fried or boiled	Same as noon meal—leftovers reheated
or	Potatoes	Bread, biscuits, or cake
Baked beans	or	Tea
Bread with margarine, jam, or molasses	Hash (salt meat and potato)	*Occasional Foods*
Tea	or	Eggs, two or three times weekly
Between Meals (Two or Three Times Daily)	Thick soup (stew)	Dried peas, cooked tomatoes with macaroni, once weekly
Bread, biscuits, jam, cake, or cookies	or	Evaporated milk (1 to 2 tsp.) in tea
Tea	Boiled dinner (potatoes and turnips; occasionally cabbage and carrots; salt or fresh meat if available)	
	Bread with margarine, jam, or molasses	
	Tea	

The preparation of vegetables makes it doubtful whether much of the vitamin content is retained. Both meat and vegetables are cooked for long periods of time. The supply of fruits is limited to a few oranges during the spring and summer months, apples and dried fruits, which are used sparingly except in jam or deserts, and wild berries, also used in jam. In most instances the bread is homemade from unenriched white flour. Breakfast cereals are usually cream of wheat or rolled-oat porridge cooked overnight and served with sugar or molasses. The consumption of milk, even among children, is low. Of the 54 children studied, 4 drank no milk, 21 used milk only with tea or cereal, 21 used from one-half to one cup a day, and only 8 drank over one cup of milk daily.* A small number of persons had food prejudices, tomatoes, dark bread, and milk being the most frequent.

An attempt was made to calculate the daily intake of various nutrients from the dietary histories, using the tables of the Committee on Food Consumption

*During the winter months all schools that apply are supplied with powdered milk free by the government for the children in the school.

TABLE II. NUTRIENT INTAKE ESTIMATED FROM DIETARY HISTORY

	FAMILY	CASE	CALORIES	PROTEIN (GM.)	CALCIUM (GM.)	IRON (MG.)	VITAMIN A (I.U.)	ASCORBIC ACID (MG.)	THIAMINE (MG.)	RIBOFLAVIN (MG.)	NIACIN (MG.)
Adult women	27	62	2000	69	0.4	11	1,300	41	0.8	0.9	11
	30	73	2200	64	0.6	10	1,600	35	0.9	1.1	8
Children 10-14 years of age	28	66	1600	51	0.3	6	600	18	0.5	0.7	7
	23	50	1800	52	0.4	11	2,000	41	1.1	1.0	10
Children 3-6 years of age	30*	74	1400	42	0.6	6	1,200	26	0.6	1.1	5
	12†	25	1300	42	0.3	4	700	6	0.4	0.7	4

*No cod-liver oil.
†Cod-liver oil (?).

TABLE III. SYMPTOMS SUGGESTIVE OF NUTRITIONAL DEFICIENCY

SYMPTOMS	NUMBER OF CASES		
	WOMEN (39)	CHILDREN (24) 10-14 YR.	MEN (16)
Eyes			
Burning or itching	20	3	5
Photophobia	17	4	3
Lacrimation	14	4	7
Visual fatigue with blurring	7	1	1
Redness	6	0	4
Discharge	3	0	2
Night blindness	5	0	3
Snow blindness	2	2	9
Lips, tongue, gums			
Burning painful tongue	14	1	2
Cracked painful lips	8	7	6
Sores at angles of mouth	3	0	0
Bleeding gums	20	6	3
Cardiovascular and respiratory			
Exertional dyspnea	14	0	3
Palpitation	12	0	3
Precordial pain	3	0	0
Edema	1	0	0
Frequent upper respiratory infections	5	0	3
Gastrointestinal			
Hemorrhoids with bleeding	10	1	2
Constipation	8	3	1
Anorexia	6	0	3
Indigestion	13	1	7
Chronic diarrhea	1	1	1
Weight loss	2	0	1
Neuromuscular skeletal			
Numbness and tingling	11	1	7
Syncope or dizziness	10	1	3
Muscle weakness	6	0	2
Burning feet	2	0	3
Leg cramps	13	1	4
Painful joints	5	0	1
Skin			
Easy bruising	15	0	3
Burning	2	0	0
General			
Nervousness and irritability	14	1	3
Easy fatigability	10	0	2
General weakness	3	0	1
Anxiety	4	0	0
Insomnia	1	0	0
Depression	1	0	0

of the National Research Council. This was possible in only a small number and even in these the figures represent rough approximations. Findings representative of the dietary data are given in Table II. The figures given for the ascorbic acid content of the diet are probably much too high because of losses in preparation of food. Since the dietary calculations were subject to such gross error, they were not used in the appraisal of nutritional status.

Symptoms.—The pertinent symptoms are listed in Table III for all groups except children between the ages of 3 and 6 years, in which group symptoms could not be evaluated. Burning and painful eyes, visual fatigue, photophobia, and lacrimation were frequent complaints. All persons were familiar with the terms night blindness and snow blindness, and of the 79 adults and older children questioned, 8 gave a history of night blindness and 13 of snow blindness. Soreness of the tongue had been noted by 17 persons and cracked painful lips by 21. Bleeding gums had been present in 29 individuals, more than one-third of the group. There was a high incidence of exertional dyspnea and palpitation as well as complaints of numbness and tingling of the extremities, syncope, and dizziness. Cramps in the legs, nervousness and irritability, and easy fatigability were common complaints of the women. "Indigestion" or dyspepsia and constipation were frequent gastrointestinal symptoms, and it is of interest that Steven and Wald[8] commented on a condition known as "Newfoundland stomach," which was characterized by these symptoms in association with many vague general complaints. They suggested that the condition might be related to thiamine deficiency.

As would be expected, the various symptoms were more prevalent in the adults.

Physical Findings.—The findings on physical examination are given in Tables IVA and IVB. Various eye signs were quite prevalent, particularly conjunctival thickening and injection and Bitot's spots. The designation "marked thickening" includes instances of pterygium and pinguecula. The term "conjunctival injection" includes an increase in the blood vessels along the equator as well as gross injection of the eyeball. An invasion of the cornea by blood vessels from the circumlimbal plexus, visible with a hand lens, was designated as a "pannus." Bitot's spots were diagnosed in 50 per cent of the adults on the basis of well-defined white or yellowish pearly elevated spots located on the scleral conjunctiva, usually near the corneal limbus. The incidence of all of the eye signs increased with the age of the individuals examined, and injection of the conjunctiva was much higher in adult men than in adult women.

One of the most frequent signs observed was some change in the papillae of the tongue; this was present in over 75 per cent of the 113 persons examined. Slight changes included either minimal enlargement and redness of the fungiform papillae or slight atrophy of the papillae at the tip or sides of the tongue. Moderate to marked changes occurred in more than 20 per cent of the men and children and in more than 50 per cent of the women. A magenta-colored tongue was observed in 12 per cent of the men, 21 per cent of the children, and 51 per cent of the women. Angular stomatitis was found in 12 per cent of the men, 19 per cent of the children, and 28 per cent of the women.

Lesions of the teeth and gums were very common. Dental caries was found in over 80 per cent of persons, being high even in children 3 to 6 years of age; however, caries and missing teeth cannot be evaluated by American standards as there is no preventive dentistry in Norris Point, and when symptoms are severe or caries is extensive, extraction of teeth is the rule. The incidence of periodontal disease was high and increased with age. Gums which bled on touch-

TABLE IVA. PHYSICAL SIGNS SUGGESTIVE OF NUTRITIONAL DEFICIENCY

SIGNS	NUMBER OF CASES			
	WOMEN (39)	CHILDREN (58)		MEN (16)
		3-6 YR. (34)	10-14 YR. (24)	
Eyes				
Conjunctiva thick—pigmented				
Slight	2	5	5	1
Moderate	17	4	3	9
Marked	19	1	2	6
Bitot's spots	19	1	4	8
Conjunctival injection	21	6	3	14
Circumcorneal injection	9	5	5	9
Pannus	4	0	1	3
Xerosis	0	0	1	0
Tongue				
Papillae, atrophy and/or hypertrophy				
Slight	10	15	12	7
Moderate	8	8	3	3
Marked	15	3	2	1
Magenta color	20	7	5	2
Red tip and/or sides	5	7	3	5
Fissures	17	0	2	3
Erosions or ulcers	3	1	0	0
Serrations and swelling	1	0	0	2
Lips and mouth				
Angular stomatitis	11	6	5	2
Cheilosis	2	3	1	1
Pallor of mucous membrane	6	4	0	0
Ulcers of mouth	2	1	0	0
Teeth and gums				
Caries				
Slight	7	9	15	4
Moderate	10	6	1	4
Marked	17	11	6	5
Edentulous	5	0	0	2
Periodontal disease				
Slight	3	5	8	3
Moderate	15	0	0	8
Marked	10	1	1	3
Bleeding gums	8	1	3	4

ing were present in 21 per cent of the women, 25 per cent of the men, and 7 per cent of the children.

On examination of the skin, there were numerous abnormal findings. Changes in the sebaceous glands of the face of the type often seen in ariboflavinosis were common. Follicular hyperkeratosis was found in 36 per cent of the women, 48 per cent of the children, and 50 per cent of the men. This term includes mild "permanent goose flesh" involving merely the arms or thighs, as well as more severe and extensive lesions. Petechiae, purpuric spots, and general pallor were rarely observed.

Objective neurological findings included calf muscle tenderness and reflex and sensory disturbances in the feet and legs, although these findings were not so frequent. It is interesting to note the frequency with which systolic heart murmurs were encountered. Systolic heart murmurs have often been described in beriberi. The skeletal abnormalities which were suggestive of rickets are given at the end of Table IVB. The height of each person was recorded and the weight was taken without shoes but with the person wearing either underclothing or a bathrobe. The adults were, in general, of short stature and thin. Only one woman of the group was definitely obese. The children appeared to be undersize both in height and weight, and comparison with standards of healthy white American children[13, 14, 15] indicated a trend in this direction. In Fig. 1 are

TABLE IVB. PHYSICAL SIGNS SUGGESTIVE OF NUTRITIONAL DEFICIENCY

SIGNS	NUMBER OF CASES			
	WOMEN (39)	CHILDREN (58)		MEN (16)
		3-6 YR. (34)	10-14 YR. (24)	
Skin—facial				
Seborrhea, nasolabial	7	7	5	1
Seborrhea, face, ears	5	4	2	0
Erythema	5	2	1	0
Skin—general				
Follicular keratosis	14	16	12	8
Dryness and scaling	6	1	4	0
Acneiform eruption	4	0	1	0
Thick and pigmented over elbows and knees	12	2	7	2
Purpura and petechiae	4	0	0	0
Bluish-red, cold extremities	5	3	4	8
Neuromuscular				
Calf tenderness	5	0	0	3
Absent knee jerk or ankle jerk	3	1	0	1
Plantar dysesthesia	3	0	0	2
Motor weakness (legs)	1	0	0	0
Absent or decreased vibratory sensation	2	0	0	3
Cardiovascular				
Systolic murmur	23	8	4	3
Pitting edema	0	0	0	0
Skeletal				
Frontal or parietal bosses		10	0	
Protuberant abdomen		7	0	
Harrison's groove		6	2	
Knock-knees		6	0	
Wrist joints enlarged		2	0	
Enlarged costochondral junctions		2	6	
Winged scapula		1	3	
Flaring ribs		1	1	
Scoliosis		0	1	

given the findings for boys. It can be seen that the weight of the Newfoundland boys aged 3 to 6 years was within the normal range of the American group but that 8 of 19 children were of shorter stature than 80 per cent of healthy American boys. In the group 10-14 years of age, 3 of 10 boys were underweight and 7 were underheight according to these American standards. Somewhat similar findings were recorded among the girls, as indicated in Fig. 2. It should be noted that none of the children were above the ranges in height or weight. The intercristal diameter was measured in the children aged 3-6 years and was found to be within the range of normal American children as given by Vickers and Stuart.[13]

In an appraisal of general attitude and mental and emotional reactions, it was observed that both women and children were extremely shy and often apathetic. The children, especially, lacked spontaneity and were inactive and quiet. There was no enthusiasm or exuberance of spirit and little inquisitiveness was manifest during the examination procedure. Children appeared older than their years as far as facial expression was concerned. Very little humor was exhibited, and in adults a lack of initiative with an attitude of acceptance of difficulties was the rule. Apathy in the children and the paucity of ambition and "pep" were noted by Mitchell[6] in her studies in Newfoundland. However, the lack of organized play may be a contributing factor to these conditions.

Laboratory Findings.—Hemoglobin determinations were made in 37 women and 42 children. In Table V are given the findings as well as the criteria used in judging the degree of anemia. The incidence of anemia, as might be

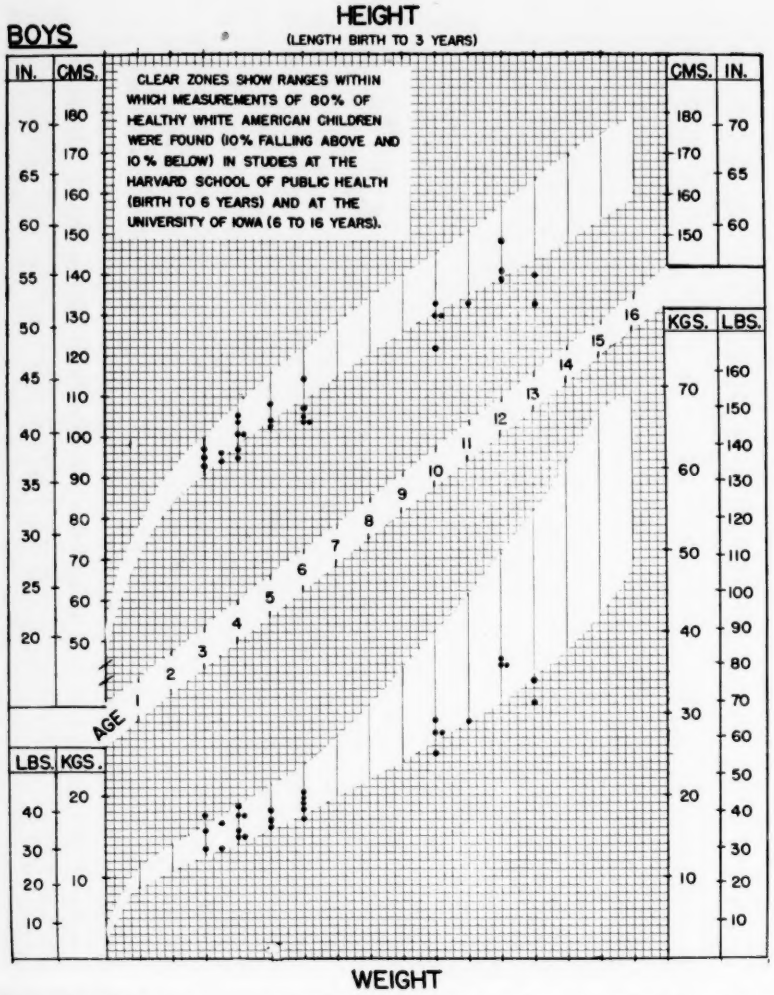
BOYS
HEIGHT
(LENGTH BIRTH TO 3 YEARS)
CLEAR ZONES SHOW RANGES WITHIN WHICH MEASUREMENTS OF 80% OF HEALTHY WHITE AMERICAN CHILDREN WERE FOUND (10% FALLING ABOVE AND 10% BELOW) IN STUDIES AT THE HARVARD SCHOOL OF PUBLIC HEALTH (BIRTH TO 6 YEARS) AND AT THE UNIVERSITY OF IOWA (6 TO 16 YEARS).
IN.
CMS.
AGE
LBS.
KGS.
WEIGHT

Fig. 1.—Height and weight of Newfoundland boys compared with American boys.

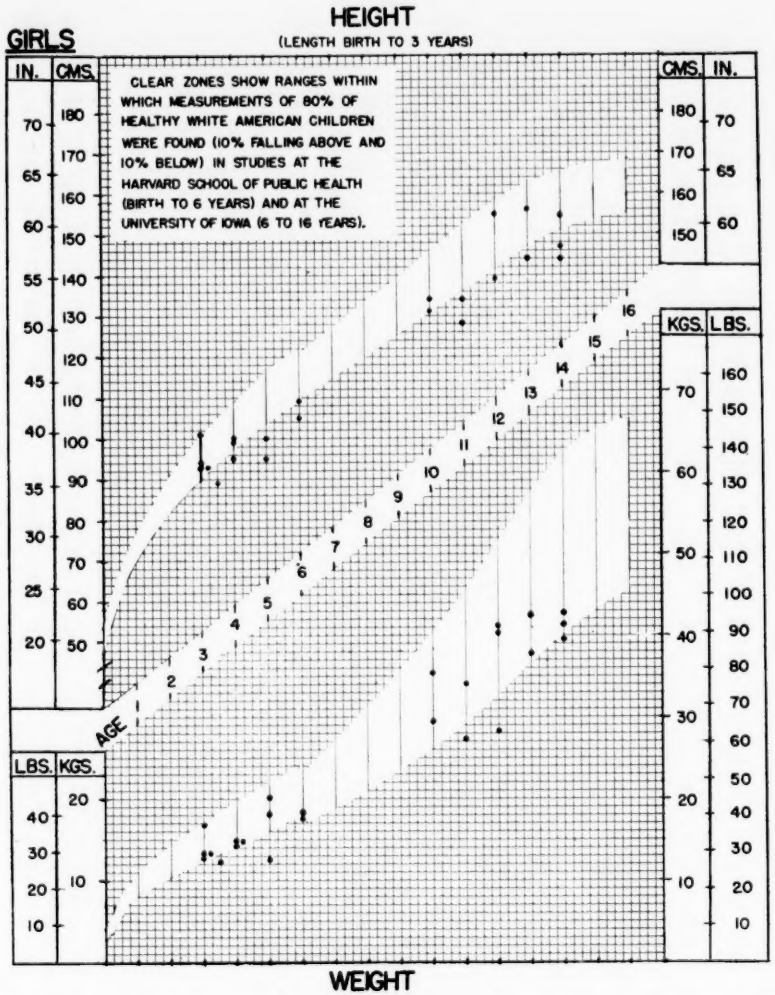
GIRLS
HEIGHT
(LENGTH BIRTH TO 3 YEARS)
CLEAR ZONES SHOW RANGES WITHIN WHICH MEASUREMENTS OF 80% OF HEALTHY WHITE AMERICAN CHILDREN WERE FOUND (10% FALLING ABOVE AND 10% BELOW) IN STUDIES AT THE HARVARD SCHOOL OF PUBLIC HEALTH (BIRTH TO 6 YEARS) AND AT THE UNIVERSITY OF IOWA (6 TO 16 YEARS).
IN.
CMS.
AGE
LBS.
KGS.
WEIGHT

Fig. 2.—Height and weight of Newfoundland girls compared with American girls.

TABLE V. INCIDENCE OF ANEMIA AS INDICATED BY HEMOGLOBIN DETERMINATIONS

	WOMEN (37)			CHILDREN 3-6 YR. (21)			CHILDREN 10-14 YR. (21)		
	HEMOGLOBIN (GM. %)	NO. OF CASES	PER CENT CASES	HEMOGLOBIN (GM. %)	NO. OF CASES	PER CENT CASES	HEMOGLOBIN (GM. %)	NO. OF CASES	PER CENT CASES
Severe anemia	< 10.0	1	2.7	< 9.5	0	0.0	< 10.0	0	0.0
Moderate anemia	10.0-11.5	11	29.7	9.6-10.5	1	5.0	10.1-11.0	2	9.5
Slight anemia	11.6-12.5	10	27.0	10.6-11.5	7	35.0	11.1-12.0	2	9.5
No anemia	> 12.5	15	40.5	> 11.5	13	60.0	> 12.0	17	81.0

anticipated, is highest in the women, 60 per cent of the sample, while 40 per cent of the younger and 19 per cent of the older group of children had subnormal levels of hemoglobin.

Plasma protein determinations were carried out in 38 of the women and 43 of the children. The results are given in Table VI. A protein level of less than 6.0 Gm. per cent was considered suggestive of hypoproteinemia, and this was found in 6 children and 3 women.

TABLE VI. PLASMA PROTEIN VALUES

GROUP	GRAMS PER CENT OF PLASMA PROTEIN					
	<5.5	5.5-5.9	6.0-6.4	6.5-6.9	7.0-7.4	7.5+
Women (38)	0	3	7	18	9	1
Children 3-6 yr. (21)	1	5	9	5	1	0
Children 10-14 yr. (22)	0	0	4	13	5	0

DISCUSSION

An attempt was made to determine the frequency with which nutritional deficiency occurred in the sample by evaluating symptoms, objective physical signs, and laboratory data. All of the symptoms and signs listed in Tables III, IVA, and IVB and some of the laboratory data in Tables V and VI are suggestive of various types of inadequate nutrition, although none alone is pathognomonic for a nutritional deficiency. They must be interpreted together and evaluated in terms of the individual as a whole. As a convenient and conservative way of applying the data obtained in the diagnosis of deficiency disease, a purely arbitrary classification was set up and each individual was diagnosed as having apparent, suggestive, questionable, or no deficiency in regard to each of the vitamins, as well as to iron and protein. Apparent deficiency was diagnosed if the person showed three signs or two signs and one or more symptoms which are considered to be characteristic of deficiency of a given dietary substance. Suggestive deficiency was indicated when the person showed two signs, or one sign and one or more symptoms. A person was designated as having questionable deficiency if only one sign was present or if signs were less definite. Symptoms alone were not used as the basis for the diagnosis of a deficiency. Individual judgment plays a considerable role in any such classification, and some of the criteria used in the evaluation may be shown to be incorrect. Nevertheless, such an evaluation gives an estimate of the incidence of deficiency disease in the community studied.

The results are given in Table VII, and it is observed that 41 per cent of the total sample were judged to have an apparent deficiency; 37 per cent, suggestive deficiency; 13 per cent, questionable deficiency; and only 9 per cent, no deficiency. The incidence of apparent deficiency was highest in the women, next highest in the men, and lowest in the children of the 3-6-year group.

The incidence of apparent and suggestive deficiency of individual factors is given in Table VIII. Deficiency of vitamin A, riboflavin, and iron were the conditions most frequently encountered. Women showed the highest in-

Fig. 1.—Height and weight of Newfoundland boys compared with American boys. Fig. 2.—Height and weight of Newfoundland girls compared with American girls.

TABLE VII. INCIDENCE OF NUTRITIONAL DEFICIENCY—GENERAL CLASSIFICATION

GROUP	TOTAL NO. EXAMINED	APPARENT DEFICIENCY		SUGGESTIVE DEFICIENCY		QUESTIONABLE DEFICIENCY		NO DEFICIENCY	
		NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT
Women	39	24	61.5	12	30.8	2	5.1	1	2.6
Children 3-6 yr.	34	8	23.5	16	47.1	7	20.6	3	8.8
Children 10-14 yr.	24	7	29.2	7	29.2	5	20.8	5	20.8
Men	16	7	43.8	7	43.8	1	6.3	1	6.3
Total	113	46	40.7	42	37.2	15	13.3	10	8.8

TABLE VIII. INCIDENCE OF SPECIFIC NUTRITIONAL DEFICIENCIES

TYPE OF DEFICIENCY	WOMEN (39)		CHILDREN (58)				MEN (16)		TOTAL	
			3-6 YR. (34)		10-14 YR. (24)					
	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT
Vitamin A										
Apparent	9	23.1	1	2.9	4	16.7	4	25.0	18	15.9
Suggestive	15	38.5	4	11.8	6	25.0	8	50.0	33	29.2
Thiamine										
Apparent	3	7.7	0	0.0	0	0.0	2	12.5	5	4.4
Suggestive	3	7.7	0	0.0	0	0.0	4	25.0	7	6.2
Riboflavin										
Apparent	11	28.2	4	11.8	5	20.8	4	25.0	24	21.2
Suggestive	11	28.2	5	14.7	3	12.5	6	37.5	25	22.1
Niacin										
Apparent	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Suggestive	3	7.7	1	2.9	0	0.0	1	6.3	5	4.4
Ascorbic acid										
Apparent	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Suggestive	5	12.8	1	2.9	3	12.5	3	18.8	12	10.6
Vitamin D										
Apparent	–	–	2	5.9	3	12.5	–	–	5	8.6
Suggestive	–	–	7	20.6	0	0.0	–	–	7	12.1
Iron										
Apparent (Hgb <11.5 Gm.)	12	32.4	1*	5.0	2	9.5	–	–	15	20.3
Suggestive (Hgb. 11.6-12.5 Gm.)	10	27.0	7*	35.0	2	9.5	–	–	19	24.1
Plasma protein										
Apparent (<5.5 Gm. %)	0	0.0	1*	4.8	0	0.0	–	–	1	1.2
Suggestive (5.5-6.0 Gm. %)	3	7.9	5*	23.8	0	0.0	–	–	8	9.9

*Hemoglobin and plasma protein determinations were made on only 21 children in this age group.

TABLE IX. DISTRIBUTION OF MULTIPLE DEFICIENCIES

GROUP	NO DEFICIENCY	ONE DEFICIENCY	TWO DEFICIENCIES	THREE DEFICIENCIES	FOUR DEFICIENCEIS	FIVE DEFICIENCIES
Women (39)	3	8	16	7	4	1
Children 3-6 yr. (34)	10	14	8	2	0	0
Children 10-14 yr. (24)	10	4	6	2	2	0
Men (16)	2	4	5	2	3	0

TABLE X. NUTRITIONAL DEFICIENCY COMPARED WITH THE NUMBER OF PREGNANCIES

NUMBER OF WOMEN	NUMBER OF PREGNANCIES	DEFICIENCY*	NO DEFICIENCY†
13	1-3	12 (92%)	1 (8%)
16	4-6	15 (94%)	1 (6%)
10	7-12	9 (90%)	1 (10%)
Total 39		36 (92%)	3 (8%)

*Deficiency refers to the diagnosis of an apparent or suggestive deficiency in an individual mother.

†No deficiency refers to questionable and no deficiency in an individual mother.

cidence of deficiency of riboflavin and iron, while men showed a greater incidence of vitamin A deficiency. There were no instances of typical pellagra, and niacin deficiency was considered to be infrequent. We felt that in many instances an inadequate supply of niacin accompanied riboflavin deficiency, although signs suggestive of the latter dominated the clinical picture.

Thiamine deficiency as indicated by objective findings was infrequent, but symptoms suggestive of this deficiency were common; however, using the criteria arbitrarily set up, a diagnosis of apparent or suggestive thiamine deficiency could rarely be made. In addition, few men were included in the sample, and it was pointed out by Aykroyd[5] that 80 per cent of the beriberi he observed in Newfoundland occurred in men. The low incidence of ascorbic acid deficiency is undoubtedly due to the rigid criteria used in this survey. If the presence of gum changes alone was considered to be definite evidence of vitamin C deficiency, the incidence would be extremely high. If plasma or whole blood ascorbic acid values could have been determined, they probably would have been low. This would have given another objective sign and the incidence of ascorbic acid deficiency as judged by the arbitrary criteria set up would have increased considerably. Twelve children of the 58 examined had signs of old rickets. Hypoproteinemia was rarely observed except in the young children, and here it may be questioned as to whether a plasma protein value of less than 6.0 Gm. per cent is indicative of hypoproteinemia.

Many persons had a deficiency of more than one essential food factor. The distribution of multiple deficiencies is given in Table IX.

The knowledge that nutritional requirements are elevated during pregnancy and the increasing realization of the importance of good nutrition during this period make it of interest to evaluate these data with reference to the clinical incidence of nutritional deficiency in relation to pregnancy and to the age of the mother. In the sample of 39 mothers, 36 (92 per cent) had either an apparent or suggestive deficiency of any of the several types observed. The incidence of deficiency associated with pregnancy is high and remarkably constant irrespective of the number of pregnancies as shown in Table X.

The most prominent deficiencies in the adult women were vitamin A, riboflavin, and iron. Many mothers had combinations of these deficiencies. The total incidence of these deficiencies correlated with the number of pregnancies and with age of the mothers is given in Table XI. There appears to be an increasing incidence of vitamin A deficiency with additional pregnancies and in the age group over 36. Deficiency of riboflavin and iron was higher in the group with four to six pregnancies than in the group with fewer pregnancies. Deficiencies of these two nutrients were higher in the group 26 to 30 years of age.

TABLE XI. SPECIFIC NUTRITIONAL DEFICIENCIES COMPARED WITH NUMBER OF PREGNANCIES AND AGE

	NUMBER OF WOMEN	VITAMIN A DEFICIENCY*	RIBOFLAVIN DEFICIENCY*	IRON DEFICIENCY*
Number of pregnancies				
1-3	13	4 (31%)	5 (38%)	7 (58%)
4-6	16	11 (69%)	13 (81%)	11 (69%)
7-12	10	9 (90%)	4 (40%)	4 (44%)
Age group (yr.)				
18-25	10	4 (40%)	4 (40%)	5 (50%)
26-30	10	6 (60%)	9 (90%)	7 (70%)
31-36	9	5 (56%)	5 (56%)	5 (63%)
Over 36	10	9 (90%)	4 (40%)	5 (56%)
Total	39†	24 (62%)	22 (56%)	22 (59%)

*Deficiency—both apparent and suggestive deficiency are grouped in this category.
†Hemoglobin determined in 37 patients.

If one correlates the total number of all the individual deficiencies in the group of women with the number of pregnancies and age, a relationship similar to that of riboflavin and iron is obtained; that is, there was a greater incidence of all deficiencies in the group with four to six pregnancies and in the group 26 to 30 years of age. In connection with these correlations, it should be noted that the number of pregnancies increased with the age of the mother. It follows that the influence of pregnancy alone in increasing the incidence of deficiency disease cannot be ascertained from these data.

An interesting finding was noted when the family groups were studied as a unit for incidence of nutritional deficiency. In a majority of instances the same type of deficiency disease was noted in the children as was shown by the mother. In some families almost identical lesions were seen in each of the members. This was particularly true of changes in the tongue. The incidence of deficiency was highest in the children of mothers who had had five or six pregnancies (79 per cent) and next highest in the children of mothers who had had three or four pregnancies (57 per cent).

From the records of the local hospital it was possible to obtain information regarding the administration of vitamin preparations, liver, and iron to persons included in this survey. Fourteen persons had received iron; 14, some source of the vitamin B complex; 12, cod-liver oil; and 2, thiamine. Of this group of 42 persons who had received therapy, 21 had no evidence of the nutritional deficiency for which the supplements had been prescribed. A number of persons who had received no medication were also without evidence of deficiency disease; for example, 10 women had no anemia and 11 no evidence of riboflavin deficiency. It is probable that the persons treated were those with severe deficiency states and that as a result of therapy, the incidence and severity of nutritional deficiency were somewhat decreased.

A few other observations made during the survey may be mentioned. There was practically no hypertension. One man had a blood pressure of 160/90, while 2 women had blood pressures of 140/105 and 140/100, respectively. The incidence of tuberculosis is high in Norris Point, but only one of the persons examined had evidence of tuberculosis and the lesion was minimal. The death rate from tuberculous meningitis is relatively high in children. Of the women examined, 2 had diffuse and one nodular nontoxic goiter, 2 showed signs of mild hyperthyroidism, and 1 of severe hypothyroidism. Three women had symptoms suggesting chronic cholecystitis, and 1 man had a peptic ulcer. Three women gave a history of some type of toxemia of pregnancy. There were 3 instances of definite and 3 of probable heart disease among the women, 2 of heart disease in the children, and 1 in the group of men. Rheumatic fever is the usual etiologic factor in heart disease in the community of Norris Point.

SUMMARY

A survey of the nutritional status of a selected sample of recently pregnant women, their preschool and preadolescent children, and their husbands and a few additional men living in the community of Norris Point, Newfoundland, is reported. The sample was selected to represent the poorest nutrition in the community and consisted of 39 women, 34 children in the age group 3-6 years, 24 children 10-14 years of age, and 16 men. This group comprised about 40 per cent of the entire population of the village.

On the basis of medical histories, physical examination, hemoglobin and plasma protein determinations, the incidence of nutritional deficiency among the women was as follows: apparent, 61 per cent; suggestive, 31 per cent;

questionable or none, 8 per cent. Children were similarly evaluated and showed the following: preschool children—apparent deficiency, 23 per cent; suggestive, 47 per cent; and questionable or none, 30 per cent; preadolescent children—apparent deficiency, 29 per cent; suggestive, 29 per cent; and questionable or none, 42 per cent. Of the men, 44 per cent had apparent, 44 per cent suggestive, and 12 per cent questionable or no deficiency. Multiple deficiences were the rule, and those most frequently encountered were vitamin A, riboflavin, and iron in the women and children; vitamin A and riboflavin in the men. Dietary histories were taken but were not used in estimating the incidence of deficiency disease because of the inaccuracies inherent in this procedure.

A hemoglobin of less than 11.5 Gm. per cent was found in 32 per cent of the women and in 7 per cent of the children. A plasma protein value of less than 6.0 Gm. per cent was found in 8 per cent of the women and 14 per cent of the children. Marked dental caries was recorded in 44 per cent of the women, 29 per cent of the children, and 31 per cent of the men. Severe periodontal disease was noted in 26 per cent of the women, 3 per cent of the children, and 19 per cent of the men. Twenty per cent of the children had evidence of old rickets.

The findings indicate a need for remedial measures. An isolated community such as Norris Point is an ideal laboratory for investigation of the value of various therapeutic procedures.

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PARENTERAL NUTRITION

I. Studies on Fat Emulsions for Intravenous Alimentation

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PARENTERAL NUTRITION has an important place in the practice of medicine and surgery and is a phase of applied nutrition in which there is considerable interest at present. The extent to which parenteral nutrition should furnish complete nutrition depends naturally on the nutritional state of the patient and the length of time over which parenteral alimentation is to be used. For limited periods in the well-nourished individual, saline and glucose are usually adequate, but for longer periods of time and in poorly nourished patients more completeness in nutritional therapy is certainly needed.

Of the six broad classes of nutrients—protein, fat, carbohydrate, minerals, vitamins, and water—all have been given parenterally, at least on occasion. The parenteral administration of water, saline, and glucose are commonplace. More recently the water-soluble vitamins and various protein preparations (casein hydrolysates, plasma, albumin, etc.) have been used parenterally. Preparations of the fat-soluble vitamins A and D suitable for parenteral administration are available though are not widely used. As far as we know, mineral mixtures other than saline, designed to supply the body with the many inorganic elements needed in normal nutrition, have not been devised. While fat has been given parenterally, its administration by this means is quite unusual and it is generally not considered in parenteral therapy.

In a preliminary note on "complete parenteral nutrition"[1] we reported experiments in which dogs were infused with mixtures containing all the nutritional factors known to be required by that species. These infusion mixtures contained an emulsion of fat. Because the fat emulsion appeared to be one of the sources of trouble in these experiments and because we believe fat is a desirable constituent of a complete parenteral nutrition medium, studies were directed to the fat emulsion.

The chief advantage of the use of fat in parenteral nutrition resides in its contribution to the energy intake. Since oil-in-water emulsions do not exert osmotic effects, any convenient concentration of fat may be used. This, coupled with the high energy content of fat, makes it possible to introduce considerable quantities of energy into relatively small volumes of infusion mixture. The use of fat in such a mixture renders it more "physiologic" in its content of the basic foodstuffs and may thereby serve useful metabolic purposes. The lowering of the specific dynamic action of protein by the concomitant feeding of fat[2] is such an example of the dietary value of fat. In an infusion mixture fat can also serve as carrier for the fat-soluble vitamins.

There are numerous references in the literature to the intravenous use of oil-in-water emulsions. Of these perhaps only a dozen have been devoted to emulsions for intravenous alimentation, the others being devoted to metabolic, pathologic, and pharmacologic studies. The former have been briefly reviewed

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by Dunham and Brunschwig.[3] It is significant that only a few of these have been experimental studies and only in three[1, 3, 4] have experiments been described covering the daily infusion of fat for any period of time. In this paper we wish to describe the preparation of a number of fat emulsions and their effects upon dogs when given daily by infusions over varying periods of time.

EXPERIMENTAL

Emulsification.—In our early experiments[1] emulsions were prepared with a small hand homogenizer. This does not operate at sufficiently high pressures to give droplet sizes as small as those of the chylomicron emulsion (diameter, 35 mμ to 1 μ).[5] It was thought that some of the untoward effects of our early infusion mixtures might be attributed to emulsions with too great a droplet size. Accordingly, a high pressure homogenizer* was obtained which has been used for all the experiments to be described. This machine operates at pressures up to 5,000 pounds per square inch and produces emulsions of droplet size roughly comparable to that of the "bright" chylomicra. All of the emulsions described were prepared at pressures from 2,000 to 3,300 pounds per square inch since data of the Cherry-Burrell Corporation and our own experience indicate that little further reduction in particle size may be expected at pressures above 2,000 pounds.

Stabilization.—The problem of stabilization of emulsions is an extremely difficult one under ordinary conditions and the extra "breaking" impetus of sterilization by autoclaving adds to the problem. The stabilizer (a) must maintain the status quo of the emulsion from preparation through autoclaving and standing, (b) must be relatively nontoxic, and (c) its emulsions must be nontoxic. In our experience, a great number of common "stabilizers," "emulsifiers," and "wetting agents" do not efficiently fulfill the first requirement, and almost none fulfill the other two requirements. Most investigators have used egg "lecithin" as the stabilizer for their emulsions,[3, 4, 6-9] but the expense and unavailability of this substance induced us to try other materials. There are two general types of emulsion stabilizers: the surface-active or "wetting agent" type and the hydrophilic colloids. The first is believed to effect stabilization by orientation at the oil-water interphase, thereby imparting a charge at the periphery of the droplet; the second is believed to envelop the droplet with a protective film of the colloidal material. Since surface-active materials are hemolytic and generally toxic, it would appear that dangerous cumulative toxic effects might result unless the substance could be efficiently metabolized. A search was made for surface-active substances containing chemical groupings similar to those of tissue substances. "Igepon T," olyl-taurine,† was tried since it resembles the bile salt taurocholic acid in containing a fat-soluble acid in peptide linkage with taurine. Cetyl phosphoric acid, prepared by a slight modification of the method of Christensen,[10] was used in the hope that a phosphatase could reduce its toxicity. Phemerol and Phemerol octyloxyacetate,‡ and Glyceryl oleate edible A and Glyceryl mono laurate§ were not studied since they were found unsatisfactory as stabilizing agents.

Of the hydrophilic colloid group, a series of gelatins and phosphatides were studied. These included an edible gelatin,‖ two gelatins developed for intra-

*Cherry-Burrell Junior-125 Viscolizer, Cherry-Burrell Corporation, Chicago, Ill. We are indebted to Mr. P. Ziegler and Mr. C. H. Preston, of the Cherry-Burrell Corporation, Boston, Mass., for the temporary use of this machine and for technical assistance in its operation.

†General Dyestuff Corporation, New York, N. Y.

‡Parke, Davis & Co., Detroit, Mich.

§Glyco Products Co., Brooklyn, N. Y.

‖Overland Gelatin, S. S. Pierce Co., Boston, Mass.

venous use as blood substitutes,* and two pharmaceutical gelatins, Pharmagel A and Pharmagel B.† Two soybean phosphatide preparations,‡ commercial soybean lecithin and purified soybean phosphatides, were also used. Gum arabic was found unsatisfactory as a stabilizing agent and hence was not studied.

A series of corn oil (Mazola) emulsions was prepared with each stabilizer in which the pH and the concentration of the stabilizer were varied. In each case an attempt was made to find the conditions most favorable for the action of the stabilizer and thereby reduce the necessary quantity to a minimum. When an emulsion of reasonable stability and employing a minimum of stabilizer was obtained, it was used in an infusion experiment. All emulsions were autoclaved at 15 pounds' pressure for twenty-five minutes before use. The composition of seven emulsions stabilized with Igepon T, cetyl phosphoric acid, and the gelatins is given in Table I; that of five emulsions stabilized with soybean phosphatides is given in Table II.

TABLE I. COMPOSITION OF EMULSIONS USED IN EXPERIMENTS 1 THROUGH 9

EMULSION NUMBER	5A	5E	6H	6J	7F	8A	8
				Grams Per Liter			
Water	925	930	925	925	881	740	890
Corn oil	67	67	67	67	105	208	89
Haliver oil	2		0.9				0.9
Igepon T							17.8
Cetyl phosphoric acid			2.67	2.67			
Pharmagel A					9.5		
Pharmagel B						13.5	
Gelatin (S. S. Pierce)	6.0						
Knox gelatin P6-00		9.4					
NaCl	1.89	1.89	1.87	1.87			3.89
KCl	1.85		1.85				2.47
Na_2HPO_4	2.26	3.02					1.37
$NaH_2PO_4.H_2O$		1.96	0.72	0.38	0.07		0.15
Tartaric acid					0.8		
$NaHCO_3$						4.5	
NaOH			2.0	2.0	0.6		
pH	7.6	6.9	7.4	7.4	8.1	8.5	7.4

TABLE II. COMPOSITION OF EMULSIONS USED IN EXPERIMENTS 10 THROUGH 16

EMULSION NUMBER	10B	10D	11H	11J	11M
			Grams Per Liter		
Water	870	870	800	800	800
Corn oil	100	100	150	150	
Coconut oil					150
Commercial soybean lecithin	30	20			
Purified soybean phosphatides			36	18	27
Na_2HPO_4	4.14	2.76	8.0	4.0	6.0
pH	7.25	7.25	7.6	7.6	7.6

Infusion Experiments.—Normal adult dogs were used in all of the experiments, and they were maintained on a Purina dog chow ration fed ad libitum. In all experiments except 1 and 2 they were trained to lie down on an animal table, and the fat emulsion was given from a Murphy drip bottle into a leg vein in the usual fashion. In the first two experiments the dogs were subjected to continuous infusion from an apparatus similar to that described by Jacobs.[11] All emulsions not prepared isotonic were rendered so at the time of infusion by the addition of suitable quantities of sodium chloride solution. Infusion Experiments 1 through 9 are summarized in Table III; Experiments 10 through 16, in Table IV.

*Knox Gelatin Co., Johnstown, N. Y., and Upjohn Co., Kalamazoo, Mich.
†Pharmagel Corporation, New York, N. Y.
‡American Lecithin Company, Elmhurst, Long Island, N. Y.

DISCUSSION

From the results given in Table III, it can be seen that all of the emulsions used in Experiments 1 to 9 inclusive were highly toxic. Infusion of emulsion 8, which was stabilized with Igepon T, resulted in death of the animal (Experiment 1) in three days, although infused very slowly. The cause of death was in all probability the result of extensive pulmonary infarction. The vomiting and excessive salivation observed also indicated toxicity, perhaps due to the surface-active stabilizer. These symptoms were noted when emulsions stabilized with Igepon T were infused into dogs in our earlier studies employing the hand homogenizer. Emulsions stabilized by cetyl phosphoric acid produced fatal pulmonary embolism in Experiments 4 and 6 (emulsions 6H and 6J). In Experiment 3, emulsion 6H produced thrombophlebitis of such extent that the experiment had to be terminated. In Experiment 6 an infusion of 95 c.c. produced fatal consequences on the first day of the experiment. Emulsions 6H and 6J were markedly hemolytic in vitro. It is interesting that comparable quantities of cetyl phosphoric acid dispersed in water do not produce such effects when injected intravenously. Evidently much of the toxic effect of this substance depends on its orientation at an oil-water interphase. The high toxicity of these emulsions is interesting in view of the fact that, owing to its efficiency as a stabilizer, relatively small quantities of cetyl phosphoric acid were used in their preparation.

Emulsions stabilized with the various gelatin preparations caused pulmonary embolism (Experiments 2, 5, 7, 8, 9, Table III). There was little variation in the pattern of symptoms. Labored abdominal breathing, salivation, vomiting, and defecation occur in rapid succession within fifteen minutes to one hour after beginning the infusion. If the infusion is not stopped at this stage, death may occur. The gelatins are well tolerated when infused alone in a solution of

TABLE III. SUMMARY OF INFUSION EXPERIMENTS 1 THROUGH 9

EXPERIMENT	EMULSION	STABILIZER	WEIGHT OF DOG (KG.)	SEX	DURATION OF EXPERIMENT (DAYS)	AVERAGE DAILY AMOUNT USED (C.C.)	AVERAGE NUMBER CALORIES PER KG. FROM FAT PER INFUSION	AVERAGE INFUSION TIME (MIN.)	GROSS EFFECTS OF INFUSION
1	8	Igepon T	13.8	F	3	450	26	880	Vomiting; excessive salivation; death
2	5A	Gelatin (Overland)	12.3	F	2	500	24.5	950	Vomiting; salivation; convulsions; pulmonary embolism; death
3	6H	Cetyl phosphoric acid	13.6	M	2	118	5	120	Experiment stopped—all veins thrombosed
4	6H	Cetyl phosphoric acid	16.2	M	2	137	5		Pulmonary embolism; death
5	5E	Gelatin (Knox) P6-00	10.3	M	3	232	13.5	101	Coughing, abdominal respiration, and defecation during all infusions—stopped experiment
6	6J	Cetyl phosphoric acid	12.5	M	1	95	4.5	65	Vomiting; defecation; urination; pulmonary embolism; death
7	8A	Pharmagel B		F	1	25		15	Vomiting; defecation; and labored breathing—stopped experiment
8	7F	Pharmagel A		F	1	64		22	Vomiting; defecation; urination; salivation; labored breathing—stopped experiment
9	5A	Gelatin (Overland)		F	1	60		20	Vomiting; labored breathing—stopped experiment

the same pH and in quantities considerably in excess of that used in the emulsions. Indeed, the Knox gelatin P6-00 used in emulsion 5E is actually prepared as an infusion colloid. These emulsions all showed a tendency to aggregation into clusters of droplets when mixed with plasma in vitro; this undoubtedly accounts for the pulmonary disturbances following infusion. The gelatin-stabilized emulsions demonstrate that toxic emulsions may be prepared from relatively nontoxic stabilizers and oils.

Pharmagel A and Pharmagel B are prepared from acid- and alkali-treated precursors, respectively, and have isoelectric points in the pH ranges 7 to 8 and 4.7 to 5.0.[12] It follows that emulsion 7F stabilized with Pharmagel A at pH 8.1 must have a gelatin film about isoelectric or perhaps slightly negatively charged. The Pharmagel B stabilized emulsion (8A) must have a negatively charged gelatin film. Since plasma proteins are negatively charged at blood pH, there would appear to be no charge incompatibility between these emulsion films and the plasma proteins. Electrophoretic evidence indicates that the chylomicron emulsion particles are negatively charged.[5] The behavior of these emulsions is therefore probably not associated with this factor.

The toxicity of emulsions prepared with these stabilizers induced us to return to commercial soybean lecithin which had been used in our early experiments.[1] It was found that such emulsions were much more stable when prepared with the high pressure homogenizer than with the hand homogenizer. Infusion of emulsion 10B (Experiment 10, Table IV) was much better tolerated, although a transient urticaria developed during the infusion on the sixth day and was much more marked during the infusion on the seventh day. The urticaria involved the skin of the head, causing a general swelling around the face, and also involved the skin of the abdomen. There was considerable hemolysis attending the infusion of this emulsion. Emulsion 10D contained less of the soybean lecithin but likewise produced an urticaria on the first day when infused in another dog (Experiment 11, Table IV). The use of the commercial soybean lecithin as stabilizer was then discontinued. At this time, a purified preparation of soybean phosphatides was made available to us.* This preparation consists of about equal parts of soybean lecithin, cephalin, and lipositol. It is relatively free of soybean oil and free fatty acids. It is a straw-colored stable powder which is readily soluble in water and is a fairly efficient stabilizer above pH 7.2.

Emulsions 11H, 11J, and 11M were prepared, using these purified phosphatides as stabilizer and used in Experiments 12 to 16 inclusive. These emulsions were much better tolerated than any used previously and were therefore infused for longer periods of time. The dogs used in these experiments were sacrificed with nembutal on the day following the last fat infusion. Terminal findings for these dogs are given in Table V.

Experiment 12 was of the longest duration (forty-eight infusions) and throughout the experiment a total of 1.35 kg. of corn oil was infused into the dog. A progressive loss of weight and slight anemia attested to the mild toxicity of this emulsion (11H). However, the animal seemed normal in other respects. Liver function as judged by bromsulfalein elimination, plasma phosphatase, plasma cholesterol components, and plasma protein concentration appeared to be normal (Table V). The methods employed in measuring liver function have been described elsewhere.[13] It is to be noted that the bromsulfalein elimination in this animal is not as efficient as that of the control puppies in the choline deficiency studies,[13] but in all of the dogs of this series it was far superior

*We are indebted to Dr. Albert Scharf, of the American Lecithin Co., Elmhurst, Long Island, N. Y., for this and other soybean phosphatide preparations.

TABLE IV. SUMMARY OF INFUSION EXPERIMENTS 10 THROUGH 16

EXPERIMENT	EMULSION	STABILIZER	SEX	DURATION OF EXPERIMENT (DAYS)	TOTAL NUMBER OF INFUSIONS	AVERAGE DAILY AMOUNT USED (C.C.)	AVERAGE NUMBER CALORIES PER KG. FROM FAT PER INFUSION	AVERAGE INFUSION TIME (MIN.)	INITIAL WEIGHT (KG.)	FINAL WEIGHT (KG.)	INITIAL HEMATOCRIT (%)	FINAL HEMATOCRIT (%)	INITIAL HB. (GM. %)	FINAL HB. (GM. %)	GROSS EFFECTS OF INFUSIONS
10	10B	Commercial soybean lecithin	F	8	8	198	10.5	126	16.7	16.7	42.0	27.5	13.3	11.3	Urticaria developed by sixth day; occasional shivering, rapid breathing
11	10D	Commercial soybean lecithin		1	1	58	2.5	30	20.7						Immediate urticaria
12	11H	Purified soybean phosphatides	F	55	48		13.6	130	19.5	17.0	37.0	32.5		9.9	Loss of weight; mild anemia
13	11H	Purified soybean phosphatides	M	29	27	200	16.0	141	17.7	16.55	48.0	34.0	14.6	10.5	Loss of weight; mild anemia
13a			M	39	0				16.55	18.1	34.0	42.0	10.5	13.3	Normal after rest period
14	11J	Purified soybean phosphatides	F	29	28	167	16.5	125	13.5	13.85	32.5	29.8	12.1	10.5	None
15	11M	Purified soybean phosphatides	F	29	28	196	16.2	147	15.95	16.75	37.9	36.7	13.3	12.3	None
16	11M	Purified soybean phosphatides	M	30	29	202	15.6	153	15.75	19.2	30.5	36.1	12.1	11.3	None

TABLE V. TERMINAL FINDINGS IN DOGS OF EXPERIMENTS 12 TO 16 INCLUSIVE

	EXPERIMENT				
	12	13	14	15	16
Bromsulfalein test (μg dye per c.c. plasma in 8 minutes)	12	10	11	16	7
Plasma phosphatase (μg phosphorus liberated per c.c. plasma in 24 hr.)	205	409	148	45	57
Plasma "total" cholesterol (mg. per cent)	152	153	256	200	200
Plasma esterified cholesterol (mg. per cent)	114	128	157	138	108
Plasma Proteins (Gm. per cent)	5.3		4.7	5.2	5.35
Total liver lipids (per cent dry weight)	18.9	12.8	14.7	18.8	15.6
Total lung lipids (per cent dry weight)	17.1		19.0	16.9	16.8
Total kidney lipids (per cent dry weight)	16.2			19.5	16.8
Total spleen lipids (per cent dry weight)	16.5		11.1	18.0	9.2
Total pancreas lipids (per cent dry weight)			39.0	24.7	22.5

to that of the choline-deficient puppies. Post-mortem examination showed no gross abnormalities with the exception of numerous old renal infarcts. Microscopically, stainable fat was present in phagocytes in the lungs and in liver parenchymal and Kupffer cells. In these organs and in the kidneys a cellular reaction to this stored foreign lipid had evidently occurred, giving rise to scattered, discrete, occasionally partially necrotic granulomatous lesions. These

consisted of central lipid-laden macrophages and occasional giant cells with surrounding collars of lymphocytes. In addition there was evidence of moderate but widespread deposition of blood pigment in reticulo-endothelial phagocytes.

The visceral organs showed normal lipid contents as measured by chloroform extraction. Infused fat does not, therefore, accumulate in these organs. We have found that it is not excreted in the urine. The fate of the mass of corn oil infused in emulsion form is a question of considerable importance. It appears doubtful that any quantity would be excreted by way of the gastrointestinal tract. The conclusion seems inescapable that (1) it is deposited in the fat depots and/or (2) it is burned for energy. Careful gross observation made it doubtful that the dog in Experiment 12 had 1.35 kg. of total body fat. It seems likely, therefore, that the infused fat was utilized for energy. This conclusion is in harmony with those reached from respiratory quotient studies following infusion of fat emulsions into dogs,[14] depancreatized dogs and rabbits,[15] and human infants.[16] It is not in agreement with the conclusions of Dunham and Brunschwig[3] who failed to observe (1) consistent changes in the character of depot fat toward that of the infused fat, and (2) reduction of nitrogen excretion on low caloric intakes during fat infusion periods. The first criterion of utilization is a precarious one; the second would not be expected to be valid during active hemolysis as was the case in their dogs. To us the conclusions of these investigators seem unwarranted. For the present, however, evidence for utilization of infused fat is not unequivocal. Further studies on this question are now in progress.

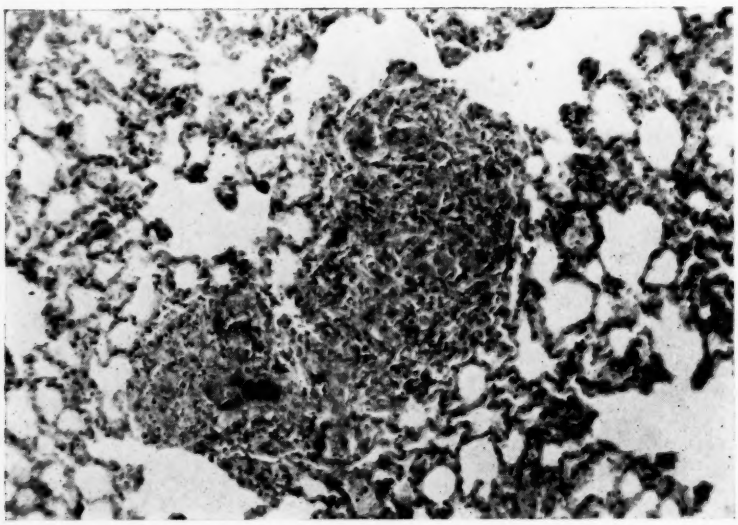

Fig. 1.—Photomicrograph of lung section (Dog of Experiment 16) showing noncaseating granulomatous lesion. Note the giant cell (hematoxylin and eosin ×100).

In Experiment 13, emulsion 11H produced a slight but significant loss in weight and a reduction in hemoglobin and hematocrit over a shorter period of time. This dog was not sacrificed until thirty-nine days after the infusions were stopped (Experiment 13a, Table IV). During this interim, its weight and blood picture returned to normal. Post-mortem studies revealed lesions similar to, but not as extensive as, those seen in the previous experiment. It is to be noted, however, that this dog received far fewer infusions than did the animal of Experiment 12.

In an attempt to reduce the toxicity of emulsion 11H, two other emulsions were prepared. Emulsion 11J (Table II) contained only half as much stabilizer as emulsion 11H and was used in Experiment 14 (Table IV). This animal showed a slight gain in weight after twenty-eight infusions and appeared to be tolerating the infusions better than the dogs receiving emulsion 11H. Terminal liver function and other findings were normal (Table V). It is apparent that part of the untoward effects are due to an excess of the phosphatides. The effects of the oil are not to be overlooked. Koehne and Mendel[17] found marked differences in the utilization of different oils given parenterally. Coconut oil was found to be superior to cod-liver oil, butter, and peanut oil in this respect. Accordingly, another emulsion, 11M, was prepared which contained coconut oil* instead of corn oil (Table II). This emulsion was used in Experiments 15 and 16. Both of the animals in these experiments tolerated the emulsion very well and appeared normal after twenty-eight and twenty-nine daily infusions. Particularly interesting was Experiment 16 in which an undernourished dog was brought back to normal during the infusion period. Its net gain was 3.45 kg., representing a gain of 21.9 per cent of the original body weight. The terminal liver function and the lipid contents of visceral organs of both dogs receiving emulsion 11M were normal (Table V). However, in the lungs of all of these animals phagocytosis of fat and a noncaseating cellular response to the foreign lipid were evident (Fig. 1).

COMMENT

These experiments clearly demonstrate that the stabilizer is a prime factor in determining the tolerance of an animal to an emulsion. All of the emulsions used in Experiments 1 to 14 inclusive were emulsions of corn oil with comparable particle size and pH. The differences in tolerance must have resulted from differences in the stabilizers employed. Of the series of stabilizers we have used, only the soybean phosphatides stand out as suitable for this purpose. If an oil-in-water emulsion is to be used as a carrier for fat-soluble vitamins, or drugs, or to absorb toxins or for any other purpose where relatively small amounts are needed, a number of other stabilizers may be satisfactory. However, for the purpose of introducing enough fat to meet requirements for parenteral nutrition, a toxicity restriction is imposed on many stabilizers and their emulsions. The literature on this subject gives but few accounts of fatal outcome following the infusion of emulsions. Perhaps the chief reason is that very small quantities have been used in most of the experiments. Dunham and Brunschwig[3] state that nine of twenty-four dogs used in their studies died because of the infusions. Their emulsions were stabilized with egg phosphatides and "Demal." McKibbin and co-workers[1] reported fatal outcomes in preliminary experiments on complete parenteral nutrition, but this cannot be attributed simply to the soybean phosphatides or their emulsions since other factors may have operated in experiments of this kind. As far as we are aware, no other investigators have reported fatal terminations of fat emulsion infusion experiments. Whether or not egg lecithin or egg phosphatide preparations may be toxic under certain conditions is an important point raised by the studies of Dunham and Brunschwig. One might expect egg and soybean phosphatides to have much in common, although there are rather marked differences in their composition. The egg phosphatides contain lecithin (2 molecules of beta to 1 of alpha), cephalin, and usually considerable cholesterol. The soybean phosphatides consist of lecithin (2 molecules of alpha to 1 of beta), cephalin, and lipositol. What

*Refined coconut oil, E. F. Drew & Co., Inc., Boonton, N. J.

differences, if any, are inherent in the lecithins other than their alpha to beta ratios, in the cephalins, in contaminating impurities, and in the general physiologic properties of their mixture are obscure. It is of interest that we have had no fatalities in a total of eleven dogs receiving emulsions stabilized with the soybean phosphatides. However, smaller amounts of soybean phosphatides were used in our emulsions than the egg phosphatides used by Dunham and Brunschwig in their emulsions. As to relative efficiency as a stabilizer, little can be said at present. Emulsions 11H, 11J, and 11M all show small but visible droplets of oil at the surface after autoclaving. The small droplets tend to float on the liquid surface in the infusion bottle and gather in the last few cubic centimeters of emulsion where they may be discarded. Hence the larger droplets are not infused. The emulsions are probably not stable indefinitely. In all of our experiments we have used emulsions made up every two weeks. Under these conditions, we have observed no outward signs or symptoms of fat embolism. It is possible that the emulsions could be used after much longer times. More work needs to be done before the relative usefulness of the egg and soybean phosphatides in this capacity can be determined. From the standpoint of availability, general stability, and ease in handling, the advantage thus far seems to lie in the direction of the soybean phosphatides.

Coconut oil appears to be superior to corn oil as an infusion fat. It is relatively nonirritating and when its emulsion is infused subcutaneously (when the needle works out of the vein, as happens occasionally), there is no local tissue reaction as is the case with the corn oil emulsions. This is similar to the findings of Koehne and Mendel.[17] With corn oil this reaction may produce swelling and soreness of the entire area, rendering the leg useless for further infusion for a week or more. One instance of peripheral neuritis appeared to result from such an accident. This constitutes a practical advantage in the use of coconut oil. A number of properties of coconut oil may be desirable for parenteral nutrition mixtures and may make it superior to many other common oils. It is almost entirely saturated, having an iodine number of 6 to 10, and would thus be much less susceptible to oxidative rancidity than other oils. It has a greater proportion of low molecular weight fatty acids (very high saponification number) and soluble fatty acids (relatively low Hehner number). These factors would be expected to increase the rate of utilization of infused fat. Experimental studies designed to evaluate the various oils or fats as to their relative suitability for this purpose are needed.

The histologic examinations reported in these studies show that even the best emulsions (11J and 11M) were not entirely inert over the time they were infused. The available evidence suggests that the foreign lipid is phagocytized by macrophages chiefly in the lungs and then gives rise to a cellular reaction resulting in the formation of noncaseating submiliary granulomatous lesions not unlike tubercles. The sequence of the changes producing these lesions and their subsequent fate cannot be determined with the material at present available for study. Lesions of this kind have not been reported by other investigators following infusion of fat emulsions.

The total energy content of all of the emulsions stabilized with the purified soybean phosphatides (11H, 11J, and 11M) was approximately 1,350 calories per liter on the basis of the fat alone. The energy furnished by the phosphatides themselves, if any, would augment this figure. On the basis of 1,350 calories per liter, it would be possible to give an "average fat intake" to a man weighing 70 kg. (750 calories or 25 per cent of the total caloric intake) with an infusion

volume of 555 c.c. It would be possible to increase the fat concentration of the emulsions should this be desirable.

SUMMARY

Corn oil emulsions of fine particle size have been prepared with a number of different stabilizers. These have included Igepon T, cetyl phosphoric acid, edible gelatin, pharmaceutical gelatins, infusion gelatins, commercial soybean phosphatides, and purified soybean phosphatides. Emulsions prepared with all these stabilizers except the last two produced severe or fatal reactions when infused into dogs in amounts sufficient to equal the usual caloric intake of fat or less. Emulsions stabilized with the commercial soybean phosphatides produced hemolysis and urticaria, whereas those stabilized with the purified soybean phosphatides produced only minor changes and these after relatively long periods of daily infusions. Refined coconut oil emulsions stabilized with the purified soybean phosphatides have so far been the more successful in infusion studies.

We are indebted to Dr. Sidney Farber and Dr. Frank Dutra for assistance with the pathologic studies and to Mrs. E. G. Patterson for technical assistance.

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TIMING OF PHAGOCYTIC CHANGES IN MALNUTRITION

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IN OUR previous reports[1, 2] on phagocytic changes in the malnutrition of vitamin or protein deficiency, we stated that the animals were kept on the deficient diets from four to seven weeks before being tested for phagocytic efficiency. Preliminary observations had indicated four weeks as the minimal time for full effects of the malnutrition to develop. We wish now to report a more detailed study of the time relationships involved.

Weanling white rats (Sprague-Dawley males) were placed in tropical warmth (90 to 91° F. and 60 to 70 per cent relative humidity) and kept on synthetic diets for eight months before being used for the study. The rats in one group received the optimal diet for tropical warmth described previously,[1] while those in the other group received a diet moderately deficient in protein and all the B vitamins (Table I).

While this low vitamin rat diet would appear to be only mildly deficient, it was about as low as would be tolerated by eight-month-old rats. Further reduction of thiamine from 1.2 mg. per kilogram down to 1.0 mg. per kilogram resulted in typical severe deficiency symptoms and death within four to five weeks. Rats of this age, kept since weanlings in the heat on the optimal hot room diet, also develop fatal thiamine deficiency in about the same time if the dietary thiamine is reduced to 1.0 mg. per kilogram.

Using the technique previously described, we measured the phagocytic activity of the blood polymorphonuclear neutrophiles and then placed the rats with deficiencies on the optimal diet while changing some of the normal rats to the deficient diet. Estimates of phagocytic activity and weight change were made weekly thereafter for each group. Four rats in each group were bled from the heart and discarded from the study at the end of each week so that the study would not be complicated by any possible effect on phagocytosis from repeated bleedings.

In Table II is set forth the changes in phagocytic activity and body weight which took place from week to week. Even though the dietary shifts in each case were promptly reflected in body weight changes within the first week, no corresponding alterations were found in activity of the blood phagocytes. By the end of the second week a moderate change was observed in phagocytic function; this became more marked during the third week and was complete by the end of the fourth week (Fig. 1). After four weeks, the rats that formerly had deficiencies exhibited normal phagocytic activity, while those previously normal were now at the low level of full deficiency.

With bacterial counts made in 40 phagocytes from each rat (making 160 cells for each mean value recorded in the table), differences in one day's readings greater than 1.5 become statistically significant. Naturally the readings of one week can be compared with those of another week only by reference to each week's normal values obtained on the control rats. The bacterial suspension used in the third week's tests was slightly too dilute, while that of the fourth week was distinctly heavy. This necessitated the calculation of

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TABLE I

AMOUNTS OF B VITAMINS AND CASEIN PER KILOGRAM OF DIET

	OPTIMAL DIET FOR TROPICAL WARMTH	DEFICIENT DIET
Thiamine hydrochloride	2.5 mg.	1.2 mg.
Riboflavin	4.0 mg.	1.5 mg.
Pyridoxine	4.0 mg.	1.5 mg.
Calcium pantothenate	6.0 mg.	1.5 mg.
Nicotinic acid	25.0 mg.	10.0 mg.
Choline chloride	5.0 Gm.	1.0 Gm.
Inositol	1.0 Gm.	0.4 Gm.
P-aminobenzoic acid	0.3 Gm.	0.1 Gm.
Casein, vitamin-free	180.0 Gm.	120.0 Gm.

TABLE II

WEEKLY CHANGES IN PHAGOCYTIC ACTIVITY

CONTROL RATS ON FULL DIET (MEAN NUMBER OF BACTERIA PER CELL)	TIME ON NEW DIET (WEEKS)	RATS CHANGED FROM FULL TO DEFICIENT DIET		RATS CHANGED FROM DEFICIENT TO FULL DIET	
		MEAN NUMBER OF BACTERIA PER CELL	PER CENT OF NORMAL	MEAN NUMBER OF BACTERIA PER CELL	PER CENT OF NORMAL
6.12 ± 0.36	1	6.59 ± 0.33	108	2.65 ± 0.18	43
6.17 ± 0.37	2	5.04 ± 0.27	82	4.10 ± 0.31	66
5.37 ± 0.33	3	2.51 ± 0.20	47	5.51 ± 0.28	103
7.57 ± 0.37	4	2.87 ± 0.19	38	8.31 ± 0.29	110
	Weight changes first week	− 10 Gm.		+ 17 Gm.	
+ 16 Gm.	4 weeks	− 68 Gm.		+ 61 Gm.	
340 Gm.	Initial average weight	346 Gm.		169 Gm.	

comparative changes on a percentage basis, using the normal controls as 100 per cent in every case. Differences of ± 10 per cent are of no definite significance here.

One review journal has recently[3] criticised our use of only 40 cells per rat (four rats to each group) as the basis for calculating mean ingestion rates, referring to the custom of counting ingestion in from 200 to 500 cells in human phagocytic studies. To test the stability of our ingestion values, we selected two representative groups from our previous report,[1] Groups 1 and 3 of the riboflavin series in the cold room. In addition to the mean values calculated on counts from 160 cells per group (40 per rat), we recalculated the data on the basis of including only the first 20, 10, 5, and 2 phagocytes seen on each slide. In Table III is set forth the results of this recalculation, and it is clearly indicated that the observed differences in mean ingestion counts maintain their statistical significance when as few as only the first 10 cells per rat are included in the calculation. It may thus safely be accepted as true that counts made on 40 phagocytes per animal, with four animals to each group,

TABLE III

NUMBER OF CELLS COUNTED		GROUP 1		GROUP 3		GROUP DIFFERENCE IN MEANS	SIZE OF DIFFERENCE IN MEANS NEEDED FOR SIGNIFICANCE (P.E. × 4)
PER RAT	PER GROUP	MEAN NUMBER BACTERIA INGESTED PER CELL	STANDARD DEVIATION OF MEAN	MEAN NUMBER BACTERIA INGESTED PER CELL	STANDARD DEVIATION OF MEAN		
40	160	5.76 ± 0.27	5.12 ± 0.19	9.37 ± 0.28	5.28 ± 0.20	3.61 ± 0.39	1.56
20	80	5.23 ± 0.38	5.03 ± 0.27	8.88 ± 0.37	4.97 ± 0.26	3.65 ± 0.53	2.12
10	40	4.55 ± 0.52	4.92 ± 0.37	8.10 ± 0.51	4.77 ± 0.36	3.55 ± 0.73	2.92
5	20	6.20 ± 0.86	5.72 ± 0.61	7.90 ± 0.85	5.63 ± 0.60	1.70 ± 1.21	4.84
2	8	6.25 ± 1.46	6.12 ± 1.03	6.75 ± 1.14	4.79 ± 0.81	0.50 ± 1.85	7.40

allow an ample margin of stability. In human case studies, 100-cell counts would provide sufficient stability if the technical details of the method were carefully standardized.

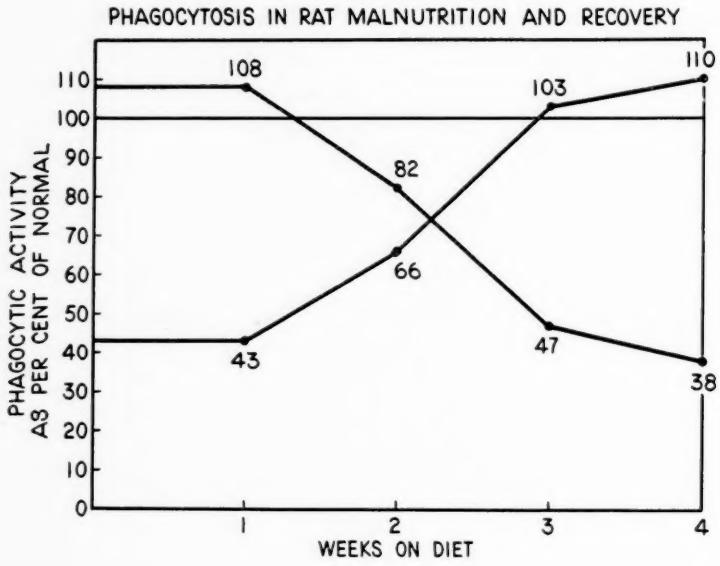

Fig. 1.

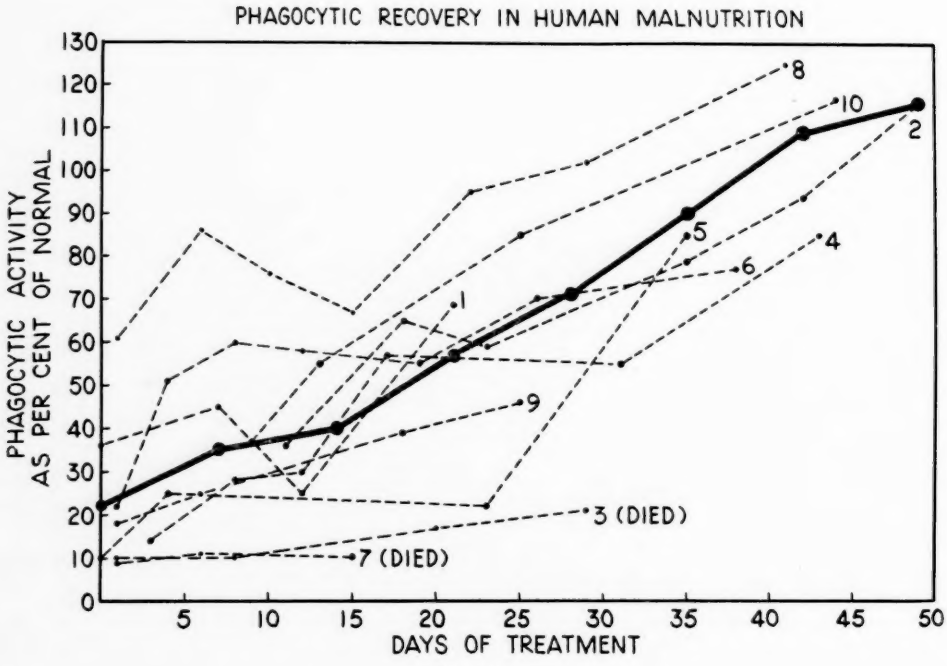

Fig. 2.

PHAGOCYTOSIS IN HUMAN MALNUTRITION

We wish also to report preliminary phagocytic studies in a few selected cases of human malnutrition. Several aged patients suffering from bacillary dysentery of variable duration were admitted to the Cincinnati General Hospital from a low-cost private nursing home. All were severely emaciated. The essential features of these cases are presented in Table IV, along with

TABLE IV

HUMAN CASES OF MALNUTRITION STUDIED

CASE	HOSPITAL NUMBER	SEX	COLOR	AGE (YR.)	DIAGNOSIS	DIET	THERAPY VITAMINS
1	162292	F	W	63	Nicotinic acid deficiency; arteriosclerosis	Regular	Nicotinamide
2	185743	M	W	36	Acute pellagra; chronic alcoholism; peripheral neuritis	Regular	Nicotinamide, thiamine, yeast
3	176787	M	C	40	Miliary tuberculosis; resection of jejunum (died)	Various	Nicotinamide, thiamine, ascorbic acid, CLO
4	175791	F	W	86	Acute enterocolitis, paratyphoid B; avitaminosis; senility	High protein High caloric, soft	Thiamine, riboflavin, nicotinamide, ascorbic acid
5	169544	F	W	80	Acute bacillary dysentery	High protein High caloric, soft	None
6	166581	M	W	73	Acute bacillary dysentery; senility	High protein High caloric, soft	None
7	45506	M	W	76	Acute bacillary dysentery; B vitamin deficiency (died)	High protein High caloric, soft	Thiamine, nicotinamide, ascorbic acid
8	182754	M	W	74	Acute bacillary dysentery; vitamin B deficiency	High protein High caloric, soft	Thiamine, nicotinamide, riboflavin, ascorbic acid
9	186064	M	W	68	Acute bacillary dysentery; malnutrition	High protein High caloric, soft	Thiamine, nicotinamide, riboflavin, ascorbic acid
10	160549	M	W	33	Beriberi heart disease	Regular	Thiamine, yeast, nicatinamide

those of three ward cases of vitamin deficiency and one emaciated patient in whom jejunal resection had been performed (miliary tuberculosis found at autopsy). All the patients with dysentery received succinyl sulfathiazole in adequate dosage, in addition to the special diet.

Phagocytic activity in the blood of these patients was estimated by comparison with two healthy normals as controls, and these normal individuals were at different times checked against healthy rats kept on an adequate synthetic diet to assure constancy.

The average rate of phagocyte recovery in this series of patients with malnutrition was slow during the first two weeks in the hospital, while from the third week onward it was more rapid and remarkably regular (heavy line in Fig 2). It is impossible to say how much of this recovery in the patients with dysentery was due to the succinyl sulfathiazole, how much to the rich diet, and how much to the vitamin therapy. The two patients receiving no vitamin supplementation made about average recovery in phagocytic function. In the first three patients with relatively uncomplicated vitamin deficiencies, good recovery of phagocytic function followed specific vitamin therapy as the only treatment administered. It is perhaps significant that the two patients who died showed very low phagocytic function at all times.

DISCUSSION

The general recovery rate in the human patients was somewhat slower than in the rats on synthetic diets, perhaps partly because the rats were other-

wise healthy, while other abnormalities retarded recovery in the human patients. One significant point of the study is the demonstration that malnutrition, from whatever cause, can produce a very marked lowering in phagocytic function. A second point of importance is the finding that phagocytic recovery follows restoration of nutritional adequacy in both rats and men, but with a lag of from one to two weeks.

Presence of this definite lag in phagocytic recovery might possibly be interpreted as meaning that improved phagocytic function comes only with release from the bone marrow of new phagocytes produced under the improved nutritional conditions prevailing. In similar manner, the lag in the decline of phagocytic activity with dietary inadequacy may mean that only new cells being produced under the deficient marrow conditions will show faulty function. Mature phagocytes already in the circulating blood seem to be less affected by the change in nutritional status of the individual.

CONCLUSIONS

Rats changed from an adequate to a deficient diet show marked weight loss during even the first week, but reduction in activity of the blood phagocytes becomes evident only during the second week and is completed by the end of the fourth week. Those changed from a deficient to an adequate diet show a similar timing in the recovery of phagocytic activity.

In human malnutrition phagocytic function is low. Dietary correction and vitamin supplementation result in phagocytic improvement which (on the average) follows a straight line rise from the third week onward, reaching normal levels in about five weeks.

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THE ADMINISTRATION OF PENICILLIN BY CONTINUOUS INTRAMUSCULAR DRIP

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WHEN penicillin is injected parenterally, it is rapidly removed from the blood, so that concentrations remaining in the serum at the end of two hours are extremely small.[1, 2] Because the drug is so rapidly eliminated from the body, it has been found necessary to inject penicillin intravenously or intramuscularly at frequent intervals. The method of administration most widely used at the present time involves single intramuscular injections every three or four hours, day and night. The general type of blood curve attained by this

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method is shown in Fig. 1. It will be noted that the amount of drug present in the blood serum is barely detectable during the third and fourth hours after each injection.[3, 4] Although intermittent intramuscular penicillin therapy is effective in the treatment of many severe bacterial infections,[5-8] the question may be raised as to whether intermittent peaks of penicillin concentration depress bacterial growth as effectively as does a continuous concentration of the drug.

The relative sensitivity of different bacteria to penicillin varies appreciably and may be measured in vitro.[9] It is doubtful whether favorable results can be expected in the treatment of clinical infections unless the amount of penicillin in the infected tissues is greater than the minimal concentration shown to exert an antibacterial effect in culture media.[10] If this premise be accepted, it would seem to follow that the most efficient method of administering penicillin is that which maintains continuously in the blood and tissues a concentration of drug greater than that needed to exert a bacteriostatic effect upon the offending organisms. It is logical to assume that the growth of the bacteria will escape from the action of the penicillin whenever the concentration of the drug falls below the minimal bacteriostatic level.

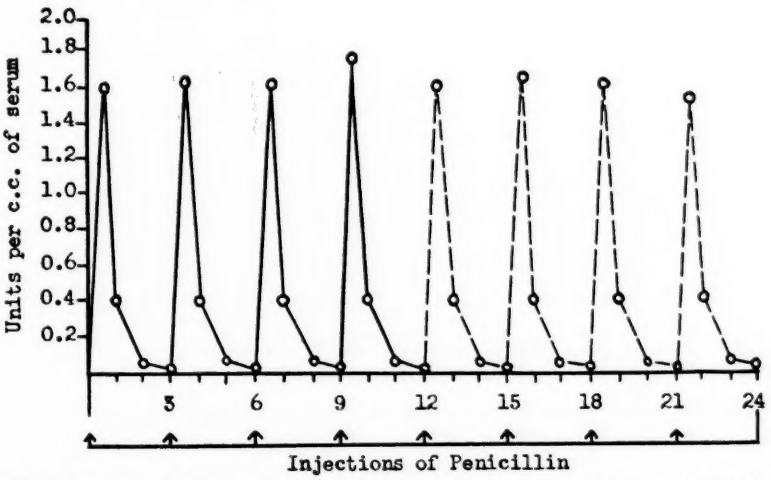

Fig. 1.—Blood level curve obtained by intermittent intramuscular injection of 20,000 units of penicillin given every three hours. Heavy lines, 0—0, represent actual observations.

Various methods have been advocated for maintaining more constant concentrations of penicillin in the blood. Some investigators have attempted to delay the absorption from the site of injection,[11-13] whereas others have administered substances which interfere with the excretion of the drug by the kidneys.[14, 15] The most promising method at the present time would appear to be that based upon the use of penicillin injected in a mixture of beeswax and peanut oil.[12]

With continuous drip therapy, absorption is slowed by the clamp on the tubing so that levels of penicillin in the blood show only small variations. Continuous intravenous drip[16, 17] has been employed but involves technical difficulties, particularly when the drug must be administered for more than a few days. Continuous subcutaneous drip has been found to be relatively unsatisfactory in that it yields significantly lower blood levels than does continuous intravenous administration.[22] Since preliminary reports suggest that continuous intramuscular therapy[3, 18-21] offers certain advantages over both of the methods just mentioned, it has seemed desirable to subject it to further clinical trial. The purpose of the present study has been twofold: first, to de-

termine quantitatively the blood levels attained by various dosages of penicillin administered by continuous intramuscular drip; and second, to evaluate the practicality of the method in the treatment of human infections.

METHODS

Administration of Penicillin.—The twenty-four-hour dose of penicillin* was dissolved in 300 c.c. of normal salt solution and administered by a standard intravenous apparatus.† Instead of the usual glass needle adapters, short metal ones were used. Tunnel clamps‡§ were employed to adjust the flow of solution to 4 to 6 drops per minute. A No. 20 standard gauge 1½-inch needle was inserted deep into the vastus lateralis muscle after preparation of the thigh with iodine and alcohol. Sterile gauze dressings were placed about the needle which was then firmly taped in place. The needle was usually left in place for two or three days before being changed to the opposite side. In one instance the needle was left in place for five days without any apparent deleterious effect. Little change occurred in the speed of flow when the clamps were once set, and it was not found necessary to watch the drip at frequent intervals.

Dosage of Penicillin.—All but one of the patients studied were given, on succeeding days, twenty-four-hour doses of 100,000, 160,000, 240,000, 400,000, and 800,000 units of penicillin. These dosage schedules corresponded to intermittent injections of 12,000, 20,000, 30,000, 50,000, and 100,000 units given every three hours. One patient, acutely ill with subacute bacterial endocarditis, was given penicillin continuously at the rate of 100,000 units every hour for a period of twelve hours.

Selection of Patients.—All but the one patient with bacterial endocarditis had early syphilis and were selected because they were relatively young and were free from significant cardiac and renal disease.

Estimation of Blood Levels.—Each test was set up as indicated in Table I. Blood serum was obtained by aseptic technique, and twofold dilutions were prepared by adding M/50 phosphate buffer.‖ A single dilution in the series was selected according to the estimated penicillin level in the serum, and varying amounts of this dilution, as indicated in Table I, were placed in test tubes measuring 1 by 10 cm. Diluent was added to each tube to bring the volume to 2 c.c.

A fresh culture of a standard strain of *Staphylococcus aureus* (209)¶ was obtained by seeding 30 c.c. of single strength broth[23] from stock cultures and

TABLE I. METHOD OF TITRATING PENICILLIN CONCENTRATIONS IN UNKNOWN SAMPLE OF SERUM (VOLUMES EXPRESSED IN CUBIC CENTIMETERS)

TUBE	BUFFER	INOCULUM	UNKNOWN
1	0	2.0	2.0
2	0.4	2.0	1.6
3	0.6	2.0	1.4
4	0.8	2.0	1.2
5	1.0	2.0	1.0
6	1.2	2.0	0.8
7	1.4	2.0	0.6
8	1.6	2.0	0.4

*The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for clinical investigation recommended by the Committee on Chemotherapeutics and Other Agents of the National Research Council.

†Manufactured by The Upjohn Co., Kalamazoo, Mich.

‡Suggested by Dr. Chester S. Keefer.

§Manufactured by The Harvard Apparatus Co., Dover, Mass.

‖A fresh pipette was used for the preparation of each dilution.

¶This strain was obtained through the kindness of Dr. J. V. Cooke.

incubating twelve hours, at 37° C. One cubic centimeter of this culture was added to 100 c.c. of double strength broth[23] and 2 c.c. of the mixture were added to each tube in the series. Aseptic precautions were observed throughout.

A single sample of commercial penicillin* containing 295 Oxford units per milligram was used as a standard. Dilutions of this sample were sealed in ampules and stored at –70° C. Repeated tests with the standard strain of *Staphylococcus aureus* (209) revealed that an amount of 0.02 units constantly inhibited growth. In each group of tests a control series of tubes was included, using the standard.

All tubes were shaken well and incubated at 37° C. for eighteen hours. Readings were made by shaking the tubes, holding them up to a strong light, and determining by inspection the last tube showing diminished turbidity (that is, bacteriostasis). In practically all instances the end point was clear as evidenced by independent readings made by several observers.

The quantity of serum in the tubes was found to have no demonstrable effect upon the end point of the test.[24] When normal human serum was substituted for the phosphate buffer, the end point was unchanged, although the bacterial growth in the tube was somewhat more luxuriant.

Since the smallest amount of serum producing inhibition of growth was known to contain 0.02 units of penicillin, the concentration of drug per cubic centimeter of serum was easily calculated.

RESULTS

Practical Advantages of Methods.—The majority of patients studied preferred the continuous intramuscular infusion to intermittent intramuscular injections given every three hours, day and night. Sleep was not interfered with, and once the needle had been taped in place, the patient was able to move about without undue discomfort. The rate of flow was not disturbed by movement of the leg, and the infusion apparatus needed only a minimal amount of attention from the nursing and resident staff. In most cases the patient was asked to watch the drip bulb occasionally and report any significant change to the nurse.

Serum Concentrations of Penicillin.—The blood levels of penicillin obtained in four patients given continuous intramuscular therapy of varying doses are shown in Fig. 2. Although some variation occurred, the blood levels attained were relatively constant throughout the twenty-four hours. The higher doses were associated with higher blood levels, and very little overlapping of the curves was observed. There was some individual variation in that all the levels of E. J. were somewhat lower than those observed in other patients. In Fig. 3 the number of units of penicillin administered in twenty-four hours is plotted against the average drug level attained in the blood. It will be seen that the levels are essentially proportional to the dosage, the relationship between the two variables being approximately linear. One patient was given penicillin continuously at the rate of 100,000 units every hour for a period of twelve hours—the equivalent of 2,400,000 units per twenty-four hours. The levels maintained in this patient's blood are shown in Fig. 4 and have been plotted separately because the magnitude of the values necessitates a different scale. Even at this extremely high dosage it was found that the linear relationship between dose and blood level still obtained.

Complications.—The reactions encountered in the sixteen patients are shown in Table II. Ten patients developed relatively severe local inflammatory re-

*Lederle Laboratories, Inc., Pearl River, N. Y.

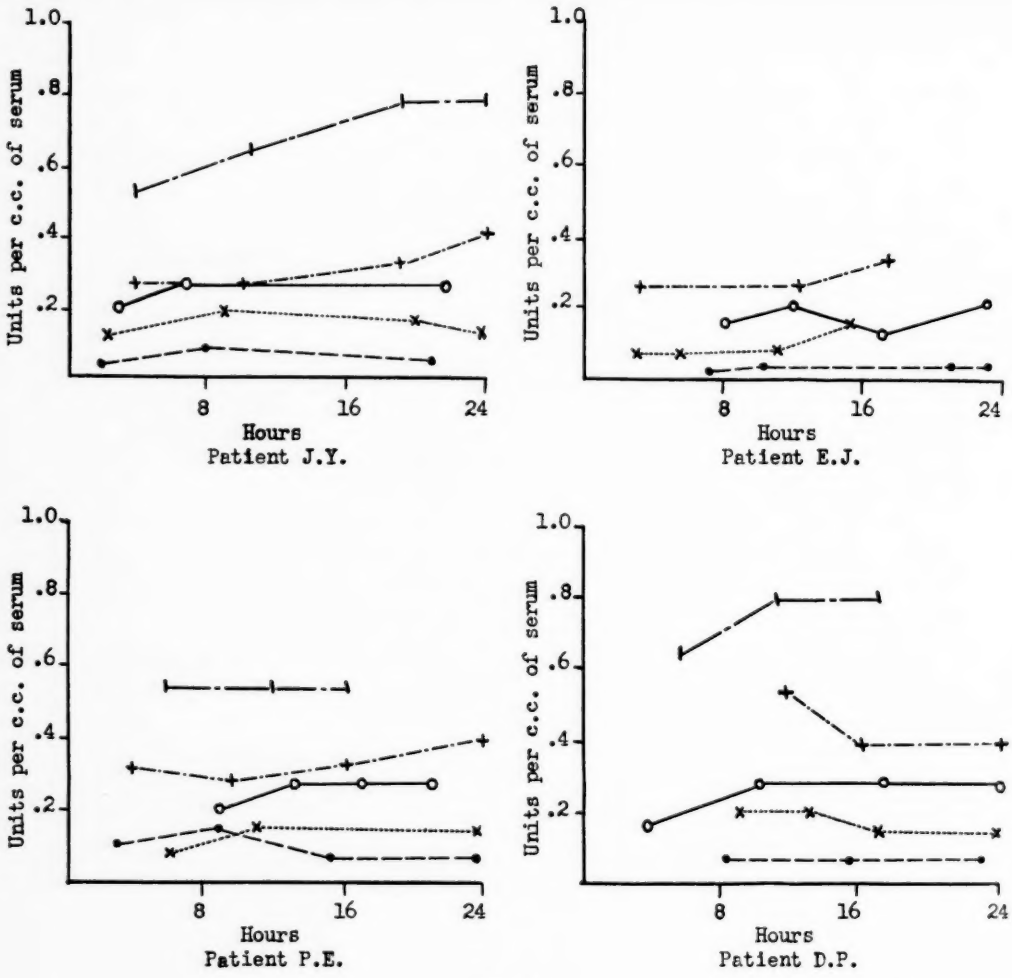

Fig. 2.

Fig. 2.—Concentrations of penicillin in the serum of patients receiving continuous intramuscular drip. Dosage in twenty-four hours: ●---●, 100,000 units; ×....×, 160,000 units; 0—0, 240,000 units; +-.-.-+, 400,000 units; |—|, 800,000 units.

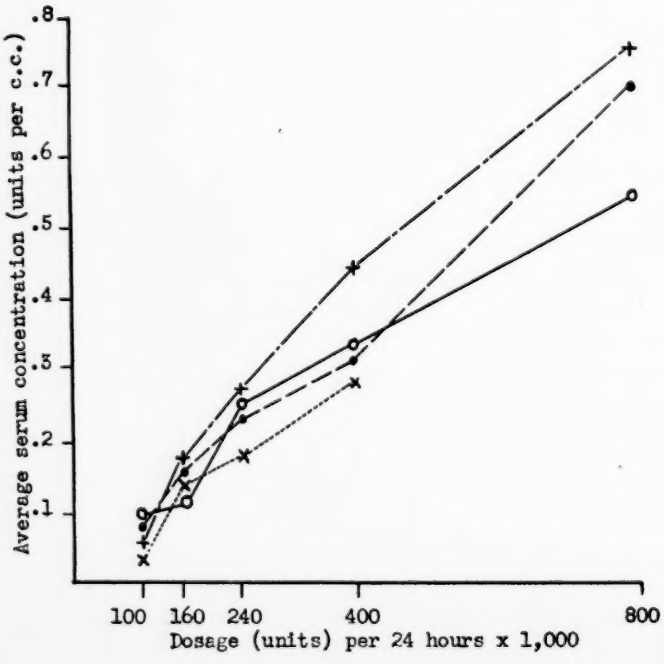

Fig. 3.

Fig. 3.—Relation of average serum level of penicillin to twenty-four-hour dosage. J. Y., ●---●; E. J., ×....×; P. E., 0—0; D. P., +-.-.-+.

TABLE II. INCIDENCE OF REACTIONS TO CONTINUOUS INTRAMUSCULAR INJECTION OF PENICILLIN

Number of patients	16
Local inflammatory reaction	10
Fever	7
Leucocytosis	5
Phlebothrombosis	1
Subcutaneous emphysema	1
No complication	5

actions at the site of injection. In all but one case the reaction developed on the fifth to seventh day of treatment (Fig. 5) and consisted of severe local pain, redness, and local heat about the site of injection, usually involving the whole lateral aspect of the thigh. The opposite thigh never showed any swelling at the site of previous injection. The inflammatory process invariably subsided rapidly (usually within twenty-four hours) when the needle was removed. No evidence of bacterial invasion of the thigh was encountered, and no local therapy was required.

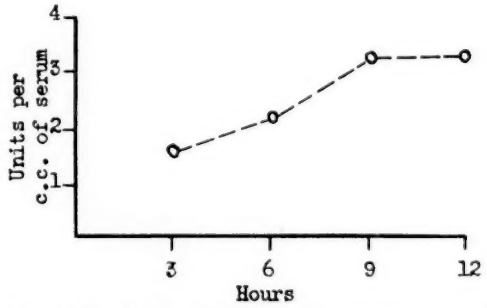

Fig. 4.—Serum levels of penicillin during continuous intramuscular administration of 2,400,000 units in twenty-four hours.

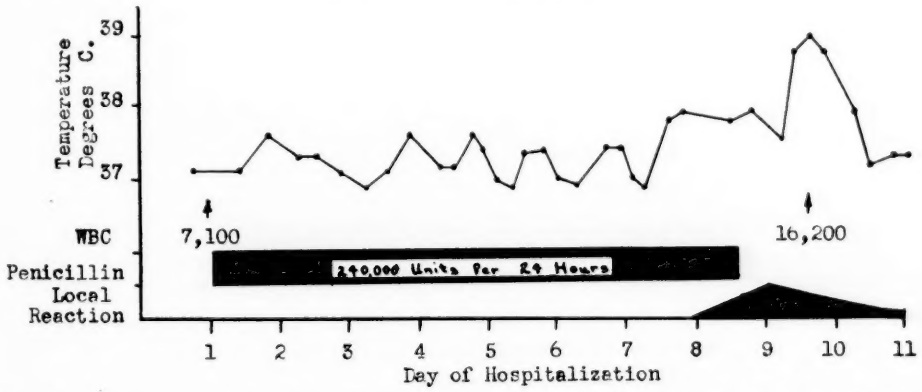

Fig. 5.—Occurrence of local inflammatory reaction, fever, and leucocytosis during continuous intramuscular administration of penicillin at the rate of 240,000 units per twenty-four hours.

The second most common reaction, which occurred in seven patients, was fever. The elevation in temperature was usually first noted on the sixth or seventh day (Fig. 5). When the continuous therapy was stopped, the fever subsided within twenty-four hours. All patients who developed fever showed evidence of a local inflammatory reaction, although the reverse was not true. The fever was frequently accompanied by leucocytosis of from 15,000 to 18,000 cells per cubic millimeter without significant shift in the differential count. In one case the fever and local reaction began on the second day, the fever ranging from 39 to 39.8° C.

A single patient developed phlebothrombosis of the femoral vein, probably as a result of inflammation of tissues above the vein. The needle in this case had been inserted into the anterior aspect of the thigh. One patient developed mild intramuscular and subcutaneous emphysema when the drip was allowed to run out, but this reaction subsided promptly.

CONCLUSIONS AND DISCUSSION

The results of the present investigation would appear to warrant two conclusions: first, that the continuous intramuscular administration of penicillin affords a practical method of maintaining relatively high concentrations of penicillin in the blood; and second, that with the penicillin preparations now available, the incidence of local reactions at the site of injection is too great to warrant routine use of the method at the present time. The blood levels attained by the continuous intramuscular drip are approximately the same as those reported with the continuous intravenous method.[22] This fact would seem to indicate that no significant amount of penicillin is destroyed at the intramuscular site of injection. It is of interest that the same linear relationship between dosage and blood level apparently obtains with both the intravenous and intramuscular routes. Blood levels as high as 2 or 3 units per cubic centimeter may be continuously maintained by the intramuscular method. It is suggested, therefore, that the method may be employed advantageously in the treatment of infections with relatively resistant bacteria.[25]

More than one-half of the patients treated in the present study developed local reactions at the site of the continuous intramuscular injections. Such local reactions have been described by previous observers.[18-20] It should be pointed out that relatively crude preparations of penicillin were used in the present study and in those previously reported. There is already suggestive evidence that the incidence of local reactions following intramuscular injections is inversely proportional to the purity of the penicillin employed.[26] It is conceivable that in the near future a crystalline form of penicillin will be available for therapeutic use. If more highly purified preparations of penicillin fail to cause local reactions at the site of injection, the continuous intramuscular method may be justifiably retained as a means of administering intensive penicillin therapy.

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ACTINOMYCOTIC INFECTION OF THE SOFT TISSUES OF THE NECK; APPARENT CURE FOLLOWING LARGE DOSES OF PENICILLIN

A Case Report

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THIS is a report of a case of actinomycosis of the soft tissues of the neck which was treated successfully with large doses of penicillin.

The patient, a white male officer, 28 years of age, was stationed in southern California. He was perfectly well until April, 1943, when he developed soreness in the region of the lower molars bilaterally. A diagnosis of impacted lower third molars was made and the teeth were extracted June 9, 1943, at his station hospital. Moderate swelling and tenderness were present on the left side following the operation but disappeared permanently after two weeks. The right side healed over a period of four weeks, but a sharply localized area of induration approximately 2 cm. in diameter remained in the soft tissues of the neck just inferior to the angle of the mandible in the posterior cervical triangle.

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There was persistent pain on motion of the right temporomandibular joint. The patient developed an ischiorectal abscess July 19, 1943. This was incised and drained five days later. On July 26, the patient's right lower jaw became so swollen that he was unable to open his mouth. This swelling partially subsided during the next four days, but an indurated area persisted unchanged in the soft tissues inferior to the mandible in the right posterior cervical triangle until December, 1943. At this time the patient noticed a gradual increase in this swelling accompanied by tenderness in the region of the maxillary gland, the floor of the mouth, and the lateral walls of the pharynx. This increased progressively until Jan. 1, 1944, when he was unable to open his mouth to eat. He was readmitted to the hospital two days later and received 52 Gm. of sulfadiazine between Jan. 3 and 15, 1944. During these two weeks the swelling greatly reduced and the range of motion in the jaw returned to normal, but a slight swelling persisted in the right submaxillary region. The swelling and induration on the right side of the jaw alternately increased and decreased in size during the next five months. He was readmitted to the hospital on May 31, 1944, and the involved area was treated with hot packs and rest. An x-ray of the jaw revealed no evidence of bone involvement. The mass in the neck gradually enlarged until it invaded the anterior cervical triangle, and July 8, 1944, a sinus developed just inferior to the middle third of the right mandibular ramus. The exudate was serous and odorless at that time. A culture taken from the sinus revealed the presence of *Staphylococcus aureus*.

A second sinus developed July 12, 1944. The mass continued to increase in size until it occupied the entire area from the angle of the jaw to the right clavicle. Seven grams of sulfadiazine were administered between July 23 and 25 without apparent change in the patient's clinical condition. The patient also received 20,000 units of penicillin intramuscularly every three hours for seventy-two hours beginning July 25. This regime was discontinued without appreciable improvement in his condition. He was transferred to Birmingham General Hospital on Aug. 4, 1944, for further treatment and disposition.

Physical examination at the time of admission revealed a well-developed, well-nourished, 28-year-old white male, in no apparent distress, not appearing acutely ill, with a brawny, indurated mass approximately 5 cm. in diameter inferior to the posterior portion of the right mandible. There were no sinuses in this area of involvement, but fluctuation was easily demonstrable. There was a similar indurated area in the anterior cervical triangle approximately 2.5 cm. in diameter with two draining sinuses. The discharge from these sinuses was serosanguineous and moderately profuse. The remainder of the physical examination was entirely within normal limits. The fluctuant mass in the posterior cervical triangle was incised and curetted Aug. 4, 1944, by Major James G. Dees.

Pus obtained from the surgically incised wound was thick, gray in color, and contained light yellow granules. These granules, when treated with 20 per cent potassium hydroxide on a slide, showed mycelia and clubbing typical of actinomycosis or ray fungus. Material was planted on Sabouraud's media, nutrient broth, and semisolid glucose agar and incubated both at room temperature and 37° C. Gram-variable fine mycelium was obtained from the nutrient broth at room temperature and 37° C., and a pure culture of fine branching mycelium was obtained on semisolid media. The growth was more abundant in the semisolid media. Some of the purulent material was centrifuged and a block made of the sediment. Hematoxylin and eosin stains showed typical

ray fungus. A culture was sent to the Registry of Fungus Diseases at Duke University. Their opinion was that it should be considered as *Actinomyces bovis*.

Penicillin was the drug of choice and it was determined to try the drug in massive doses. Small doses which had been administered during previous hospitalization, for a total of 480,000 units, had failed to produce a cure. It was finally decided that this patient would be given 7,000,000 units of penicillin over a period of fourteen days. An initial dose of 120,000 units was administered Aug. 11, 1944, followed by 80,000 units intramuscularly every four hours until the patient had received 7,000,000 units.

Course of Disease.—There was marked decrease in the swelling and discharge from the draining sinuses during the first seventy-two hours. At the end of ninety-six hours the purulent discharge completely subsided and only a thin serous discharge was present. No discharge was evident Aug. 20, 1944, and the sinuses had started to heal. The induration and enlargement of the lymph glands in the anterior and posterior triangles had decreased about 50 per cent. On Aug. 28, 1944, the sinuses and the draining incision had completely healed and only a small amount of induration remained around the sites of the former sinuses and incision. By Sept. 7, 1944, the induration around the previous sinuses and operative scar had completely disappeared and no enlarged lymph glands were found in either anterior or posterior cervical triangles.

On Jan. 4, 1945, re-examination revealed no enlarged lymph glands and there was complete disappearance of any signs of activity. The skin around the sinuses and operative scars was completely normal, and the officer was returned to full duty.

DISCUSSION

In our opinion this patient is completely cured. The very rapid and apparently complete recovery following the administration of massive doses of penicillin is strong presumptive evidence that the cure was due to the drug. Previous smaller doses and about two weeks of sulfadiazine had failed to effect a cure. The case is reported in the hope that others may have occasion to try similar doses in cases under their care. The report is also of interest in demonstrating again the comparative harmlessness of huge doses of penicillin.

NOTE.—Since this paper was written, a report has been published by Walker and Hamilton* on the successful treatment of six cases of actinomycosis with penicillin. These authors used much smaller individual doses than we did but continued the treatment over a considerably longer period of time so that the total dosage given to each of their patients approximated that which we gave our patient. In one of their conclusions they state, "The treatment with penicillin must be prolonged." The dosages given by Walker and Hamilton to their six patients were, respectively: 8,170,000 units; 5,975,000 units; 7,845,000 units; 3,360,000 units (twelve days later, 6,300,000 units during some extractions); 5,040,000 units (three weeks later, 2,520,000 units additional because of some persistent fluctuation); and 2,794,000 units (a second course of 4,300,000 units because of a recurrence). It is our belief that the key to cure of this disease with penicillin lies not in prolonged treatment but in the administration of large doses.

*Walker, John M., and Hamilton, Joseph W.: The Treatment of Actinomycosis With Penicillin, Ann. Surg. **121**: 373, 1945.

PROTHROMBIN IN PRESERVED BLOOD

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TRANSFUSION of preserved blood has proved useful, especially where donors have not been available and it has been necessary to replace blood lost through hemorrhage.

Fresh blood transfusion is a usual procedure in instances where hemorrhage is due to disturbances of coagulation; in such cases blood transfusion is most important since the blood contains all the necessary factors for normal coagulation. Should preserved blood be administered in cases of hemorrhage due to hypoprothrombinemia, it is advisable to learn beforehand whether the blood to be transfused contains sufficient amounts of prothrombin, so that the patient's blood may be given, although momentarily, its capacity for normal coagulation.[3, 8]

Several investigators have studied the prothrombin stability of preserved blood for transfusions. Generally, after some days, prothrombin gradually diminishes in the blood and hence it cannot be used for transfusions designed to correct hypoprothrombinemia; however, there is considerable disparity in medical literature regarding the speed and degree of disappearance of prothrombin in preserved blood. Thus Belk and his co-workers[1] and Lord and Pastore,[5] by means of Quick's method,[4] did not find changes in the first five days, while Quick,[6] using his own method, reported a 50 per cent decrease in the first twenty-four hours. Ziegler and his co-workers,[10] using Smith's method,[13] found that the prothrombin progressively decreases during the first thirty-six days, while Rhoads and Panzer,[7] using a modified Quick's method, found that prothrombin diminishes to 25 per cent in seven days and to 20 per cent by the end of ten days. Warner and his collaborators,[9] employing Smith's procedure, found the following values for prothrombin: 91 per cent at the end of ten days and 50 per cent within twenty-one and twenty-five days. According to these authors, the difference in results is due both to the method of preserving blood and to the anticoagulant used. We feel that the methods employed to determine the prothrombin concentration are responsible for this disparity.

EXPERIMENTATION

The blood used in this study was obtained from healthy adults in accordance with directions set by two of us[11]; that is, by mixing 9 volumes of blood with 1 volume of 0.1 N sodium oxalate (in this instance we employed sterilized sodium oxalate).

The blood thus collected was divided into two portions: one portion was aseptically stored in 2 c.c. ampules; the other was centrifuged and the separated plasma was kept in 1 c.c. ampules. These were kept, for the entire duration of the experiment, in the icebox at the Blood Transfusion Center.

Prothrombin determinations were carried out on those days indicated in Tables I and II. For fibrinogen solution, we used barium plasma prepared from the plasma of healthy human beings at the very moment the determinations were

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carried out. Thus, by diluting the plasma under study with fresh barium plasma, we were able to avoid the changes which could be attributed to the plasma itself.

In every instance, cultures were prepared from the plasma a few days after its separation; the cultures were invariably negative.

DISCUSSION

During the first week we obtained hardly any significant changes in prothrombin time* of the preserved blood samples (see Tables I and II and Figs. 1 and 3). This finding is in agreement with those of Belk and of Lord and Pastore. The prothrombin concentrations found with our method of determination are shown in Tables I and II and in Figs. 1 and 2; these, far from confirming a drop in the prothrombin concentration, show a remarkable increase which is maintained throughout the first week. From then onward a gradual increase is observed in prothrombin time and a drop in prothrombin concentration. It is interesting to point out that at the end of five months (Table I),

TABLE I. PROTHROMBIN CONCENTRATION IN A BLOOD SAMPLE PRESERVED DURING FIVE MONTHS AT 4° C.

SAMPLE 1

DAYS	BLOOD		PLASMA	
	PROTHROMBIN TIME (SEC.)*	PROTHROMBIN†	PROTHROMBIN TIME (SEC.)	PROTHROMBIN (PER CENT)
--	19.0	101	19.0	101
1	19.0	124	19.5	143
2	17.5	210	18.0	--
5	18.5	98	21.5	80
9	26.5	95	22.5	104
13	26.0	104	25.5	105
19	27.5	105	26.0	109
22	28.5	73	25.5	--
33	33.0	36	37.0	47
37	32.5	34	41.5	42
54	35.0	28	40.5	36
155	No clotting in 2 min.	30	No clotting in 2 min.	37

*0.2 ml. calcium-thromboplastin was added to a 0.1 ml. of plasma to determine the prothrombin time.

†Prothrombin concentration was determined according to the technique of Tanturi and Banfi, with three plasma dilutions.

TABLE II. PROTHROMBIN CONCENTRATION OF BLOOD AND PLASMA PRESERVED AT 4° C.

SAMPLES 1 AND 2

DAYS	SAMPLE 2				SAMPLE 3	
	BLOOD		PLASMA		PLASMA	
	PROTHROMBIN TIME (SEC.)	PROTHROMBIN (PER CENT)	PROTHROMBIN TIME (SEC.)	PROTHROMBIN (PER CENT)	PROTHROMBIN TIME (SEC.)	PROTHROMBIN (PER CENT)
--	18.5	113	18.5	113	19.5	102
1	19.0	146	19.0	130	19.0	128
2	18.0	180	19.5	190	20.0	175
3	20.0	238	17.5	180	19.5	206
4	21.5	146	21.0	126	21.0	143
6	23.0	133	20.5	103	27.0	127
7	24.0	107	23.0	102	23.0	111
8	27.0	109	30.0	98	28.0	99

*According to our technique, the prothrombin time in normal blood is not similar to Quick's because the thromboplastin we use is prepared in a different manner. Quick determines the prothrombin concentration by plotting the prothrombin time (Quick's normal is from 11 to 12.5 seconds) in a chart based on determinations carried out with different dilutions of normal plasma. Our experience has shown that this procedure may lead to considerable error, since an experimental difference of one second in the timing gives rise to great variations in the figures of prothrombin concentration.

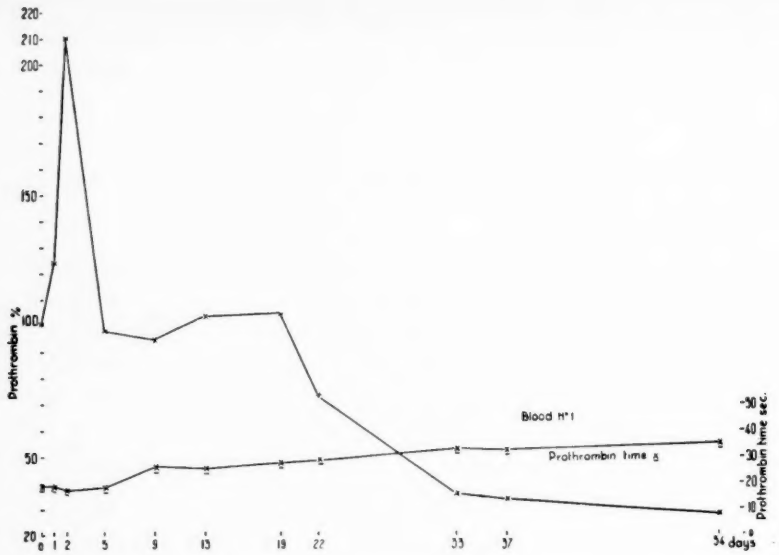

Fig. 1.—Prothrombin and prothrombin time variations in blood sample 1, stored for five months at 4° C.

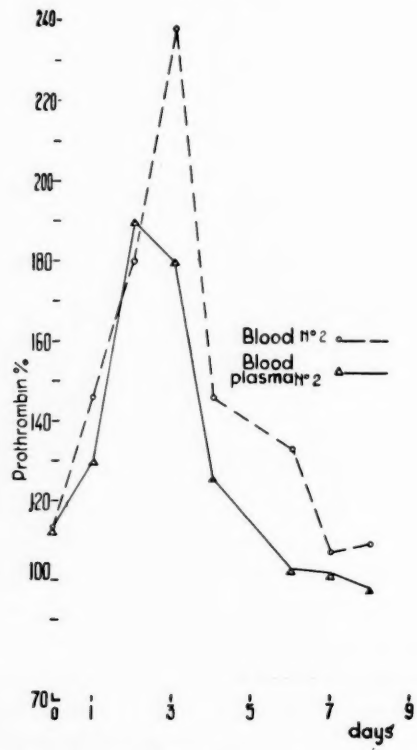

Fig. 2.—Prothrombin variations in blood sample 2, stored for eight days at 4° C.

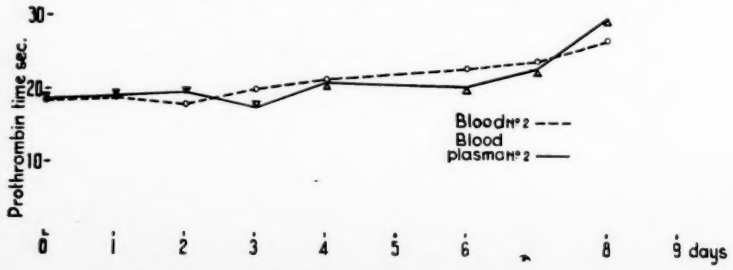

Fig. 3.—Prothrombin time variations in blood sample 2.

the plasma, although still containing 30 per cent of prothrombin, does not clot if thromboplastin and calcium are added. We attribute this noncoagulability of the plasma to an alteration or disappearance of the fibrinogen, since throughout the entire experiment we have observed the appearance of precipitates or filaments which we believe to be fibrin.

A comparative study of Tables I and II and Figs. 1, 2, and 3 shows that prothrombin time is not correspondingly shortened to the largest prothrombin concentration found when determinations are carried out with plasma dilutions, especially during the first three days of storage. The disparity in the figures is explained by the fact that prothrombin concentration does not vary in accordance with the clotting time of undiluted plasma when thromboplastin-calcium is added (prothrombin time). This is due to the fact that even the smallest experimental difference in the timing gives rise to large difference in the prothrombin concentration figures. This is more marked with concentration above 100 per cent. In fact, using our equation (constant for a given thromboplastin, at present $y = 0.57 \times -9.92$) we can calculate the prothrombin times which would correspond to prothrombin concentration above 100 per cent; thus the prothrombin time corresponding to a plasma with a 100 per cent prothrombin concentration is 19.1 seconds, while that corresponding to 300 per cent is 18 seconds. In previous papers[11] we have pointed out this source of error and have emphasized the need for carrying out determinations with dilutions of each plasma.

The increase found by us in prothrombin concentration during the first few days, or its greatest activity, cannot be attributed to modifications in the fibrinogen with which the thrombin reacts, because the diluting plasma (plasma treated with barium sulfate) was prepared each day with fresh normal plasma.

Quick[14] observed that prothrombin time increases between the fifth and ninth day of storage of oxalated plasma. He maintains that if this stored plasma is diluted with fresh alumina plasma or with plasma of a dog fed dicumarol, the prothrombin time thus obtained becomes shorter. Quick explains the previously mentioned differences by suggesting that prothrombin consists of two factors, A and B. Of these two components, A is destroyed during storage, while B, adsorbed by the aluminum, decreases in the plasma of dicumarol fed animals. When alumina plasma or dicumarol plasma is added to preserved plasma, the complex AB is re-established and the prothrombin time becomes shorter.

Quick has asked us if it would be possible to explain our results in accordance with his theory of prothrombin constitution (A and B components). To this effect we carried out a cross experiment (see Table III). The plasma of two normal subjects was studied, blood being drawn with a forty-eight-hour interval in each case. With the samples obtained on both days, diluting plasma was prepared and determinations were performed. According to Quick's hypothesis,

TABLE III. STUDY OF THE PLASMA AND DILUTING PLASMA PRESERVED FOR FORTY-EIGHT HOURS AT 4° C.

DATE		TUBE	48-HOUR PLASMA (1)	FRESH PLASMA	48-HOUR BARIUM PLASMA	FRESH BARIUM PLASMA	PROTHROMBIN (PER CENT)	
PLASMA 1	PLASMA 2						PLASMA 1	PLASMA 2
8/20	18-IX	1		x		x	111	99
		2		x	x		106	69
		3	x			x	157	212
		4	x		x		151	109
8/18	16-IX	4b	(1) This plasma was also determined at same moment of extraction and the findings were ----------				109	103

Tube 3 containing preserved plasma should have lost factor A and the addition of fresh plasma should have restored prothrombin to its normal constitution (AB complex). If Quick is right and the increase of prothrombin concentration is due to the addition of factor A, present in fresh diluting plasma and absent in stored plasma, we should never have been able to obtain the high prothrombin concentration found in our experiment; at the most, we should have had a concentration similar to that of fresh plasma.

We have already stated that prothrombin time does not vary in accordance with the prothrombin concentration (Tables I and II) and that this apparent contradiction during the first three days of storage could be attributed to an experimental error in the timing. We observe that after the fourth day prothrombin time increases gradually. This would mean a corresponding decrease of the prothrombin concentration. However, such is not the case. This discordance is possibly attributable to the formation of an antithrombin in stored plasma, as can be seen in the subsequent experiment utilizing plasma of the same subject.

TABLE IV. THROMBIN TIME IN FRESH AND STORED PLASMA

Fresh plasma	0.2 c.c. + Thrombin* 0.1 c.c.	= 22 to 24 sec.
Stored plasma (5 days)	0.2 c.c. + Thrombin 0.1 c.c.	= 43 to 44 sec.

*Thrombin prepared after Eagle[15] in a 1/10 dilution.

We have also met the influence of this antithrombin when determining the thrombin time in plasma 2 of Table III. The fresh barium plasma showed a thrombin time of 22 seconds, while in stored barium plasma the time was 32 seconds. These results would appear to explain the differences found in the prothrombin concentration of fresh and stored diluting barium plasma.

The antithrombin action becomes less important when the stored plasma is diluted with fresh barium plasma; this explains the apparent disparity found between prothrombin time and concentration during storage.

The results of the foregoing experiments lead us to suggest that the increase of prothrombin concentration is not due to substances contained in the fresh diluting plasma.

So far, no investigator has pointed out the increase found during the first few days in the prothrombin concentration of stored blood and plasma. Perhaps this increase corresponds to a greater activity of the pre-existing prothrombin, which, under the conditions of our technique, becomes manifest as a greater concentration.

Crosbie and his co-workers,[2] using Howell's method through recalcification, found a shortening of the clotting time of blood during the first few days of storage. At the same time they were able to observe a rapid decrease in the number of platelets during the first few days. It is interesting to point out a coincidence in the shortening of the clotting time, a decrease of the number of platelets in the first few days of blood preservation (Crosbie), and an increase in prothrombin concentration found by us during the same period. It is possible that the reduction in the number of platelets results in the liberation of thromboplastin. The latter would render more sensitive the existing prothrombin, thus allowing a more rapid formation of thrombin at the moment of recalcification. This hypothesis should be the subject of further studies.

CONCLUSIONS

1. During the first three days, the prothrombin time of preserved plasma and blood undergoes slight variations. From then onward the time gradually

and continuously increases until the plasma, in spite of the addition of thromboplastin and calcium, becomes noncoagulable. This final noncoagulability is not due to an absence of prothrombin.

2. The prothrombin concentration, as determined by our technique, gradually increases and reaches its "maximum" at the end of three days, when it doubles its original value; later on it gradually decreases, and by the end of five months it reaches a 30 per cent concentration. A hypothesis is put forward that this increase in concentration registered in the first few days is due to prothrombin "sensitization."

3. After a certain period, transfusion of preserved blood or preserved plasma will not correct a prothrombin deficiency.

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THE ISOLATION OF HEMOLYTIC STREPTOCOCCI FROM THROAT SWABS

II. The Efficiency of Human and Rabbit Blood in Streaked and Streaked-Poured Plates

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ALTHOUGH numerous observations have been made concerning the appearance of streptococcus colonies on various kinds of media, comparative data showing the relative value of different methods in the isolation of beta hemolytic streptococci from throat swabs are notably lacking. It has recently been shown[1, 2] that the detection of small numbers of hemolytic streptococci is facilitated by the preliminary incubation of throat swabs in an enrichment broth containing sodium azide and crystal violet. Two questions arose for which we were unable to find satisfactory answers in the literature. First, whether the number of hemolytic streptococcus isolations is materially affected by the kind of blood used, and second, whether plates which are inoculated in a manner to give deep colonies are more likely to be positive than those with surface inoculations.

The recognition of hemolytic streptococcus colonies depends largely upon observing the changes which they produce in the blood agar used for isolation. Following the demonstration of hemolysis by streptococci in rabbit blood agar by Marmorek[3] in 1902 and in human blood agar by Schottmüller[4] in 1903, it was shown that the erythrocytes of a number of different species were susceptible to hemolysis. Smith and Brown,[5] although not the first to use horse blood, based their description of alpha, beta, and gamma types on the appearance of colonies in horse blood agar. Consequently, horse blood has been considered as standard in many laboratories. Horse, human, or rabbit blood was recommended by Holman and co-workers,[6] who stated that the sharpest results were probably obtained with horse blood. Gilbert[7] reported that horse and human blood were equally satisfactory, but rabbit and sheep blood were found less suitable. Several strains from septic sore throat were hemolytic on sheep blood under anaerobic conditions only. Coffey[8] specifies either horse or human blood, while Schaub and Foley[9] state that rabbit, sheep, human or other blood may be used with little or no variation in the appearance of streptococcus colonies.

Becker[10] was one of the first to observe that strains of streptococci might be hemolytic on one kind of blood and not on others. Two strains which were apparently *Streptococcus pyogenes* were hemolytic on rabbit blood, but not on sheep or horse blood. A third strain hemolyzed horse and sheep cells but not goat, rabbit, or human cells. Smillie[11] called attention to the different types of hemolysis produced on the blood of different animals and stated that dog and guinea pig blood were not satisfactory. Brown[12] compared several strains of beta hemolytic streptococci in horse, human, and rabbit blood agar and found

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that variations in appearance on these bloods were quite insignificant. The appearance of alpha strains, however, was so dependent upon the kind of blood used that certain strains resembled the beta type on one kind of blood but not on another. Several strains of streptococci which hemolyzed human blood but not sheep blood and others which hemolyzed sheep but not human blood were recently reported by Traut and Johnson.[13] In none of these instances were the organisms identified serologically.

Sheep blood agar was used by Krumwiede and Kuttner[14] because of its inhibitory properties for the Hemophilus group. The elimination of *Hemophilus hemolyticus* colonies which often resemble those of hemolytic streptococci resulted in a medium which was partially selective for hemolytic streptococci. Human blood has a similar effect but is less inhibitory than sheep blood.[9]

It is, therefore, evident that some strains of streptococci do not produce the same appearance on plates prepared with various kinds of blood. To what extent these variations affect the efficiency of different bloods in isolating hemolytic streptococci is not clear. The kind of blood used in any laboratory depends partly upon its availability. Many laboratories do not have access to a source of horse blood. Rabbit blood is almost always obtainable, but when large quantities are required, the collection of the blood is somewhat time consuming. Human blood is, in most instances, available in the largest quantities, particularly in laboratories associated with hospitals. With the increasing prevalence of blood banks, many hospitals frequently have on hand sterile human blood which for various reasons cannot be used for transfusions.

The possible advantage of using poured plates in preference to the more convenient streaked plates for the isolation of hemolytic streptococci lacks convincing evidence. Krumwiede and Valentine[15] compared poured and streaked plates in culturing the throats of ten individuals and concluded that typical hemolysis may not be present on surface-streaked plates. The necessity of a standard method was recognized by Becker,[10] who suggested human blood-agar-streaked plates. Holman and associates[6] indicated the desirability of observing both surface and deep colonies but thought that surface inoculation was sufficient for routine purposes. Brown[12] showed that deep colonies are of more value than surface colonies in distinguishing beta, alpha, and alpha prime types. The value of poured plates in distinguishing serologic Groups A, B, and C was demonstrated by Rantz and Jewell,[16] who were able to correlate type of beta hemolysis with serologic group provided deep colonies were observed. Fry[17] observed several strains of Group A streptococci which formed nonhemolytic surface colonies when grown aerobically, but deep colonies in aerobic plates or surface colonies on anaerobic plates showed typical beta hemolysis.

Long and Bliss[18] recommended the use of poured rabbit blood plates for the isolation of minute beta hemolytic streptococci but presented no data to support the recommendation. Both surface and poured plates with horse blood were used by Rantz[19] in culturing a series of 345 excised tonsils and 298 throat swabs. Although complete data were not given, it was stated that 88 per cent of Group A strains from tonsils "were as readily isolated on surface as in poured plates." With throat swabs Rantz found that the number of isolations was influenced more by the skill of the technician than by the method of preparing the plates and concluded that skillfully prepared surface plates were nearly as satisfactory as poured plates. Dingle and co-workers[20] employed both streaked and poured plates in several hundred throat and sputum cultures but discontinued the poured plates because their use "did not increase significantly the growth of hemolytic streptococci of the beta type." No data were given.

The usual objection to the use of poured plates is that they are time consuming and inconvenient.[19, 20] Unless several dilutions of the material to be cultured are plated, there is always a risk of obtaining too many colonies for best differentiation. The necessity for pouring the plates individually at the time of inoculation is a distinct disadvantage. The first of these objections to poured plates is eliminated by the use of "double poured" plates as described by Perry and Petran.[21] In this method, thin agar plates are streaked in the usual manner and then a layer of blood agar is poured over the inoculated surface. Coffey[8] included these streaked-poured plates as an alternative to poured or streaked plates but stated that further data will be required to select the most practical method. Streaked-poured plates were tried by Rantz,[19] who found them to be satisfactory substitutes for poured plates.

It has been clearly demonstrated that the types of streptococci with reference to hemolysis can be more accurately recognized if deep rather than surface colonies are examined. It seems less certain, however, that plates prepared in a manner to produce deep colonies result in a greater number of hemolytic streptococcus isolations.

The data presented in this report provide a statistical comparison of plates prepared with rabbit and human blood and of streaked and streaked-poured plates with regard to their relative efficiency in demonstrating hemolytic streptococci on throat swabs from well children.

METHODS

Fresh beef heart infusion agar containing 1 per cent tryptose and adjusted to pH 7.6 before autoclaving was used throughout. For streaked plates blood was added in a concentration of 5 per cent. Rabbit blood was obtained by heart puncture and defibrinated by shaking with glass beads. It was stored in the refrigerator and usually was used within one week. Citrated human blood, discarded by neighboring blood banks because of positive serologic tests for syphilis, obtained in 500 c.c. amounts, was dispensed in 10 c.c. bottles and stored in the refrigerator for as long as six weeks. The amount of hemolysis which occurred in this time was not sufficient to alter the appearance of the blood agar or to interfere with the detection of hemolytic colonies.

In the preparation of streaked-poured plates, about 15 c.c. of beef heart infusion agar without blood were poured into each plate. These plates were stored in the refrigerator and were inoculated in the same manner as the streaked plates. Then 10 c.c. of melted agar containing 10 per cent blood were poured over the inoculated surface. In pouring this layer of blood agar, care was taken to disturb the inoculum as little as possible. Sometimes it was necessary to tilt the plate slightly in order to cover the bottom layer completely. A few surface colonies were desired and were always obtained, but too much mixing of the inoculum with the layer of blood agar made the colonies more difficult to fish. Best results were obtained when agar plates were prepared far enough in advance of inoculation so that the surface of the agar was dry. Experiments showed that using 5 per cent blood in both layers of agar made the plates too dense to read easily. Blood in the top layer only, in 5 per cent concentration, resulted in plates too light in color.

All plates were inoculated from overnight broth cultures of throat swabs prepared in the manner previously described.[1] Various ways of streaking plates were tried because the importance of obtaining an adequate sample of the broth culture without obscuring the results by overcrowded colonies was realized. In our hands the following method gave the desired result most consistently.

A straight needle was dipped into the well-mixed broth culture and streaked from fifteen to twenty times over about one-third of the plate. The plate was then turned 90 degrees and streaking continued at right angles to the first, again over one-third of the plate. The plate was turned again and the procedure repeated. Growth was rarely too heavy on the last section of the plate to be streaked.

Plates were examined after twenty-four to twenty-six hours' incubation at 35 to 36° C. Hemolytic colonies were fished to sections of rabbit blood agar plates. After incubation these subcultures were examined by inspection and Gram stain. Those resembling hemolytic streptococci were transferred to broth for confirmation by serologic grouping. Only one colony was fished from each plate unless the gross appearance suggested that more than one kind of hemolytic organism was present.

RESULTS

Comparison of Human and Rabbit Blood in Streaked Plates.—Broth cultures of 199 throat swabs were streaked on both rabbit and human blood plates. The results are shown in Table I.

TABLE I. COMPARISON OF RABBIT AND HUMAN BLOOD PLATES STREAKED FROM BROTH CULTURES OF 199 THROAT SWABS

	BLOOD PLATES				DIFF. %	S.D. DIFF.	P
	RABBIT		HUMAN				
	NO.	%	NO.	%			
Hemolytic streptococci isolated	66	33.2	81	40.7	7.5	4.8	0.11
Group A	26	13.1	38	19.1	6.0	3.7	0.11
Hemolytic streptococci missed	16	8.0	1	0.5	7.5	2.0	0.0001
Group A streptococci missed	12	6.0	0	0	6.0	1.9	0.001
H. hemolyticus present	10	5.0	0	0	–	–	–
Suspected colonies present	85	42.7	118	59.3	16.6	5.0	0.001
Unconfirmed colonies present	21	10.6	37	18.6	8.0	3.6	0.016
Confirmed nonhemolytic colonies	8	4.0	2	1.0	–	–	–

Hemolytic streptococci of Groups A to G were isolated from eighty-one of the human blood plates and from sixty-six of the rabbit blood plates. Group A streptococci were obtained from twenty-six of the rabbit blood plates and from thirty-eight of the human blood plates. The difference in both cases is within the range of sampling error ($P = 0.11$). The rabbit blood plates, however, failed to show hemolytic streptococci sixteen times when human blood plates from the same swabs were positive. Only once did the human blood plates fail when the rabbit blood plates were successful. Rabbit blood plates failed to isolate Group A streptococci twelve times or nearly one-third of all the A strains isolated. These comparisons of the two kinds of plates with respect to failures show differences which are statistically significant ($P = 0.0001$ and 0.001).

The reason for rabbit blood plate failures is probably not that rabbit blood provides a less favorable medium than human blood. All strains grew readily on rabbit blood after isolation. The more likely explanation is that better hemolysis on human blood made the hemolytic streptococcus colonies easier to recognize. On eight of the rabbit blood plates, nonhemolytic colonies, subsequently identified serologically, were fished because of their morphology. Only two of the human blood plates showed such colonies. Both these strains belonged to Group B.

The greater amount of hemolysis on human blood is further reflected in the fact that suspected colonies which were not confirmed as hemolytic streptococci were fished from thirty-seven of the human blood plates. Unconfirmed

fishings were made from only twenty-one of the rabbit blood plates and these included ten plates which showed *H. hemolyticus*. Even when these ten plates are included, the difference between unconfirmed fishings from rabbit and human blood plates is unlikely to have arisen from chance ($P = 0.016$).

Two factors which might have contributed to the difference in the results of using rabbit and human blood were (1) that the rabbit blood was defibrinated, while the human blood was prevented from clotting by the addition of sodium citrate, and (2) that the rabbit blood was always added to the medium within a few days after bleeding, whereas the human blood, being obtained in larger quantities, was stored for several weeks before being used. In order to control these factors, samples of human and rabbit blood were obtained, sodium citrate in a concentration of 1 per cent was added to a portion of each sample, and the remainder of each sample was defibrinated. Blood plates poured the same day the blood was obtained were streaked with pure cultures of several strains of hemolytic streptococci. After twenty-four hours' incubation, plates prepared with citrated blood were indistinguishable from those containing defibrinated blood of the same species. Hemolysis was definitely more marked on human than on rabbit blood agar.

Another difference in the appearance of Group A hemolytic streptococcus cultures on rabbit and human blood agar was repeatedly found in the morphology of the colonies. Cultures which formed flat, matt colonies on rabbit blood agar tended to be convex and glossy on human blood.

The broth containing a 1:15,000 concentration of sodium azide used for preliminary culture of the swabs did not completely inhibit *H. hemolyticus*. Since we have found *H. hemolyticus* in 41 per cent of swabs from a similar group of children cultured directly on rabbit blood agar,[2] the 5 per cent incidence here (Table I) represents a considerable reduction. Human blood [9] as well as the blood of certain animals[14] is somewhat inhibitory for organisms of the hemophilus group.

Rabbit blood plates failed most often when few hemolytic streptococci were present. The average number of hemolytic streptococcus colonies on the human blood plates from swabs which were negative on rabbit blood was eleven in comparison to an average of twenty-six on the same kind of plates from specimens which were positive on both rabbit and human blood.

Comparison of Streaked and Streaked-Poured Plates.—Streaked and streaked-poured plates were compared on 159 throat swabs (Table II). There was no significant difference between the two kinds of plates with respect to the total number of hemolytic streptococci isolated or the number which belonged to Group A. Six of the streaked plates were negative when the streaked-poured

TABLE II. COMPARISON OF STREAKED AND STREAKED-POURED HUMAN BLOOD PLATES FROM BROTH CULTURES OF 159 THROAT SWABS

	BLOOD PLATES				DIFF. %	S.D. DIFF.	P
	STREAKED		STREAKED-POURED				
	NO.	%	NO.	%			
Hemolytic streptococci isolated	62	39.0	66	41.5	2.5	5.5	>0.5
Group A	27	17.0	26	16.4	0.6	4.2	>0.5
Hemolytic streptococci missed	6	3.8	2	1.3	2.5	1.8	0.16
Group A streptococci missed	1	0.6	2	1.3	0.7	–	–
Suspected colonies present	78	49.1	83	52.2	3.1	5.6	>0.5
Unconfirmed colonies present	16	10.1	17	10.7	0.6	–	–
Average number of fishable colonies on positive plates	9	–	11	–	–	–	–

plates were positive, while only two of the latter missed hemolytic streptococci which the former detected, but this difference is probably not significant (P = 0.16). Essentially the same number of each kind of plates showed suspected colonies which could not be confirmed after isolation.

The average number of isolated colonies thought to be fishable on the positive streaked plates was nine as compared with eleven in the streaked-poured plates. The latter figure was based on deep colonies only and does not take into account the surface colonies that were frequently present. Even when both deep and surface suspected colonies were present, the former were fished in order to determine the practicability of fishing deep colonies. In no case was there a failure to get growth in subculture when deep colonies were fished. Neither was there an excessive number of impure cultures.

As would be expected, the streaked-poured plates showed larger and clearer zones of hemolysis than the streaked plates. Although this difference might be an aid in reading the plates and in predicting the serologic group, it did not, in our experience, materially increase the number of streptococcus isolations.

TABLE III. COMPARISON OF RABBIT BLOOD STREAKED PLATES WITH HUMAN BLOOD STREAKED-POURED PLATES FROM BROTH CULTURES OF 159 THROAT SWABS

	BLOOD PLATES						
	RABBIT STREAKED		HUMAN STREAKED-POURED		DIFF. %	S.D. DIFF.	P
	NO.	%	NO.	%			
Hemolytic streptococci isolated	51	32.1	66	41.5	9.4	5.4	0.09
Group A	19	11.9	26	16.4	4.5	3.9	0.23
Hemolytic streptococci missed	14	8.8	0	0	8.8	2.3	0.0001
Group A streptococci missed	7	4.4	0	0	4.4	–	–
Suspected colonies present	64	40.3	83	52.2	11.9	5.6	0.04
Unconfirmed colonies present	13	8.2	17	10.7	2.5	3.2	0.42
Positive for *H. hemolyticus*	8	5.0	0	0	5.0	–	–

Data on rabbit blood streaked plates are also available for comparison with the human blood streaked-poured plates (Table III). The difference in the number of plates showing hemolytic streptococci by these two methods is greater than the difference shown in Table I. The advantage of the streaked-poured plates with human blood over rabbit blood surface plates with respect to total and Group A streptococci isolated, however, is not statistically certain. On the other hand, no swabs were found positive by the latter which were missed by the former, while rabbit blood streaked plates failed fourteen times when the streaked-poured plates were positive. This difference appears to be highly significant (P = 0.0001). There was a difference of only 2.5 per cent in the two kinds of plates which showed hemolytic colonies not confirmed. Excluding the eight rabbit blood plates which showed *H. hemolyticus*, this difference becomes 7.5 per cent, which is probably significant (P = 0.007). The increased hemolysis due to human blood as well as that due to the presence of deep colonies probably contributed to this difference.

DISCUSSION

Although our data indicate that human blood plates are slightly superior to rabbit blood plates as far as demonstrating the presence of hemolytic streptococci is concerned, there are three points to be considered in connection with the use of human blood. (1) Cultures on human blood agar showed more suspected colonies which were not confirmed as hemolytic streptococci than did the rabbit

blood agar plates. This fact was responsible for a certain amount of unproductive work and, unless due care is exercised in confirming the identity of suspected colonies, it might lead to a number of false positive results. (2) On theoretical grounds, at least, one should avoid the use of human blood containing antistreptolysin. We did not encounter any blood which failed to show good zones of hemolysis, but the possibility should be kept in mind. (3) The difference in colony morphology of hemolytic streptococci on rabbit and human blood is probably not important as far as isolation is concerned. The possible significance of this observation in regard to variation in colony morphology is not apparent.

Our failure to demonstrate an advantage for streaked-poured plates over streaked plates using human blood does not necessarily mean that no difference would have been observed if rabbit or horse blood had been used. Since deep colonies are more hemolytic and since eight of sixty-six positive cultures showed nonhemolytic surface colonies on rabbit blood plates, one might expect that streaked-poured plates would be more efficient than streaked plates if rabbit blood were used.

It would appear that by inoculating different kinds of plates from the same broth cultures variable factors were reduced to a minimum in the three sets of data compared. In Table I the only known factor contributing to the difference was the kind of blood; in Table II, the manner of preparing the plates; and in Table III, both these variables were present. The comparisons should, therefore, be more accurate than if independent, although similar, samples of throat swabs had been treated according to the methods described.

SUMMARY

After preliminary culture in enrichment broth, 199 throat swabs from well children were streaked on both human and rabbit blood agar plates. Hemolytic streptococci were isolated from 41 per cent of the human blood plates and from 33 per cent of the rabbit blood plates. Rabbit blood plates failed sixteen times to show hemolytic streptococci when the corresponding human blood plates were positive. Only one swab was negative on human blood when the corresponding rabbit blood plate was positive. The greater efficiency of human blood is thought to be due to the tendency of streptococci to be less hemolytic on plates prepared with rabbit blood.

A second series of enrichment broth cultures of 159 throat swabs were examined in streaked-poured plates for comparison with surface streaked plates. Hemolytic streptococci were isolated from 42 and 39 per cent, respectively. This difference as well as the differences in the number of Group A streptococci isolated and the number of failures by the two methods are well within the range of sampling error. The data presented fail to reveal a definite advantage for streaked-poured plates over streaked plates using human blood infusion agar.

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PROGRESS

CLINICAL SIGNIFICANCE OF SERUM PROTEIN (ALBUMIN AND GLOBULIN) CHANGES

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AS THE result of much work during recent years it has become clear that a knowledge of the serum protein level is of considerable significance in the diagnosis and treatment of many medical and surgical conditions. Thus, important changes of the serum protein concentration may be encountered in Bright's disease, liver disease, and malnutrition, in conditions associated with plasma depletion, including burns, hemorrhage, tissue trauma, peritonitis, and intestinal obstruction, and in others. It is little wonder, then, that the clinical laboratories are frequently called upon to do serum protein determinations.

Before discussing certain specific examples of altered serum albumin and globulin concentrations encountered clinically, a brief consideration will be given to the subject of plasma protein formation and to the physiologic role of plasma protein in controlling fluid exchange between the plasma and the interstitial fluid compartment.

METABOLISM OF PLASMA AND TISSUE PROTEINS

Normal Range of Variation of Serum Proteins.—Although, for practical purposes, only the two protein components albumin and globulin are usually determined clinically, evidence points to the presence in serum of a number of different globulins and probably more than one albumin. As is well known, globulins can be separated readily from albumins by precipitation at salt concentrations above a certain point. For example, in the familiar Howe procedure[1] the globulins are precipitated by a 22 per cent sodium sulfate solution. Further, when the sodium sulfate is present at concentrations of 13.5, 17.4, and 21.5 per cent, respectively, three globulin precipitations are realized and these have customarily been termed euglobulin, pseudoglobulin I, and pseudoglobulin II. The significance of the latter globulin fractionation has been obscured somewhat by recent electrophoretic studies of serum,[2-5] which showed that aside from albumin, serum normally contains three globulin components; namely, α-, β-, and γ-globulin. Although relatively few data are available at present concerning the changes of these globulin components in health and disease, there would appear to be significant disturbances in their absolute and relative amounts in certain diseases exhibiting hyperproteinemia.[3, 6]

From the findings of several groups of workers[7, 8] it would appear that in normal adults the total serum protein concentration may vary between 6.0 and 8.0 Gm. per 100 c.c.; albumin, between 3.6 and 5.5 Gm. per 100 c.c.; and globulin, between 1.4 and 3.5 Gm. per 100 c.c. The great majority of the figures for the total serum protein concentration apparently fall within the narrower limits of 6.1 and 7.7 Gm. per 100 c.c.[6, 8, 9] The ranges of variation of serum albumin and globulin depend somewhat upon the method of estimation. Thus, Robinson, Price, and Hogden[10] found, in studying the determination of the

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albumin and globulin fractions of blood serum by the Howe method,[1] that a variable amount of albumin is adsorbed by the filter paper. It was found, further, that within rather wide limits the amount of albumin adsorbed is independent of the concentration of albumin but is dependent upon the type and quantity of filter paper. Such adsorption of albumin causes an error in the determination of the albumin concentration. Indirectly, therefore, it also causes an error in the globulin figure, since the latter is taken as the difference between the total serum protein concentration and the albumin concentration. By employing the Howe method and introducing the precautions necessary to avoid loss of filtrate protein through adsorption on filter paper, Gutman and co-workers[6] found the albumin to vary between 4.7 and 5.7 Gm. per 100 c.c. and the globulin to vary between 1.3 and 2.5 Gm. per 100 c.c. in thirty-six normal subjects. In other words, the range of values for albumin was found to be somewhat higher and that for globulin to be somewhat lower than those usually cited.

It seems well established[6] that significantly higher values for total serum protein and albumin may be encountered in healthy persons, for example, in laboratory workers and students, rather than in hospitalized control subjects. There is evidence[8] that at birth the total serum protein concentration and protein fractions, especially the globulin, are lower than those observed in adults. However, the various values rise shortly after birth so that from 3 to 6 months of age[8, 11] they fall within the normal ranges of variation for adults.

Plasma Protein Formation.—It would appear that the normal organism has a considerable reserve capacity for forming new plasma proteins since in the absence of infection or disease and in the presence of an adequate protein intake, an acute depletion of plasma protein is rather quickly corrected. Conclusive information is still lacking regarding the origin of plasma proteins, especially albumin and globulin. However, Whipple and his associates[12-15] and others[16] have presented evidence that the liver is the site of plasma protein production or, at least, is intimately concerned with plasma protein fabrication. The belief that the albumins come from liver seems quite well established. However, there still remains some doubt in the case of the globulins since, even although the liver may play an important role in contributing them, there is evidence[17, 18] that the reticulo-endothelium and other cells may also be sites of origin of globulins.

The ability of the normal organism to fabricate new plasma protein has been studied experimentally by determining the plasma protein regeneration following protein depletion induced either by plasmapheresis or by the administration of a diet very low in protein. The former method of plasma protein depletion has been employed extensively by Whipple and his co-workers.[14, 20] In this method the plasma protein of a dog on a basal diet is reduced and maintained by repeated plasmapheresis at a level close to 4 Gm. per 100 c.c. This subnormal level of the plasma protein is assumed to act as a constant and maximal stimulus for the production of new protein. The plasma protein production is then measured by determining the amount of protein which must be removed by plasmapheresis to maintain the plasma protein concentration at the reduced level. In such experiments it was found that when the plasma protein depletion was begun, the weekly output of protein was high at first and then gradually fell to a basal output. However, when the basal diet and other factors were kept uniform, the basal output of protein was found to remain relatively constant and such a standardized animal could then be employed to test the effect of diet and of other factors on protein regeneration.

The initial excess of new plasma protein over the basal output has been designated by Whipple and his co-workers as a "reserve store" of plasma protein building material. Its amount was found to vary in different animals[21, 22] and apparently depended upon the preliminary dietary intake, being low following a period of low protein intake and high following a period of increased protein intake. It was found that the reserve store of protein, suitable for plasma protein production, could be depleted not only by plasmapheresis, but by fasting or by long-continued protein-free diets as well. It was found, further, that when the reserve stores were once depleted the animal could produce scarcely any new plasma protein and new plasma protein production was very largely, if not wholly, dependent upon materials coming in from the gastrointestinal tract.[14, 20]

The studies with protein-depleted dogs have revealed a remarkable ability of the organism to regenerate plasma protein. Thus, it has been shown[23] that with a favorable protein intake an animal may be subjected to a regime of prolonged intensive plasmapheresis without any impairment in the ability to regenerate plasma protein. On the other hand, chronic or acute infection may bring about a marked reduction of plasma protein regeneration.[24, 25] That plasma protein regeneration may be markedly influenced by the amount and quality of the dietary protein was early recognized by Whipple and his colleagues. Thus, it was found that dogs receiving food regenerated plasma protein more rapidly than those fasting and, further, the regeneration was found to be more rapid on a meat diet than on a diet of milk and bread.[26, 27] Vegetable protein in the basal ration was found to be definitely inferior to meat protein,[25] while the "potency" values of beef serum, beef muscle, and casein were found to be in the order given.[25, 28] Gelatin, when added alone to the basal ration, was found to increase plasma protein regeneration only slightly, while gelatin plus the amino acid tryptophane induced a marked regeneration.[22] Zein was found to have no effect upon plasma protein regeneration, but when it was supplemented by cystine, tryptophane, lysine, and glycine, there followed marked regeneration.[42] More recently[29, 30] it has been demonstrated that a mixture of the ten purified amino acids essential for maximum growth in the rat was as effective by mouth as most dietary proteins for the production of plasma protein.

The study of plasma protein regeneration following experimental nutritional hypoproteinemia is of considerable interest clinically, since a comparable situation is encountered not infrequently in patients with lowered plasma protein levels due primarily to malnutrition. Weech and his collaborators[31, 32] found in dogs that the administration of a diet very low in protein resulted in a progressive fall of the total serum protein concentration, which at first was rapid and then more gradual. During this change, the globulin concentration was found to remain relatively constant so that the fall of serum protein represented a depletion of the albumin fraction. It was found, further, that when the period of depletion was short and the vitality of the animal was not impaired, the regeneration of albumin upon the resumption of adequate feeding was rapid and followed a path of equal daily increments until the normal concentration was attained. However, after long and debilitating periods of depletion, the regeneration, although initially rapid, was found subsequently to be retarded and the albumin to approach the normal concentration slowly. This delayed regeneration was taken as evidence pointing to an injury of the mechanism concerned in forming albumin, which is presumably located in the liver.

Weech and Goettsch[33] have presented a method of assay of dietary protein for serum albumin regeneration based upon the increase of the serum albumin concentration arising from the ingestion of a definite amount of the protein for a given interval by dogs after they were subjected to a low protein diet for a period of three weeks. These authors[34] found the order of potency for albumin regeneration of the following dietary protein sources to be beef serum, beef muscle, and casein. It will be noted that the same relative order was found, as given previously, when the plasmapheresis technique was utilized to produce plasma protein depletion.

Blood and Tissue Protein Losses With Restricted Protein Intake.—It is now clear that both the blood and tissues contribute to the total nitrogen lost from the body during periods of restricted protein intake. From the results of studies of the nitrogen balance, Weech, Wollstein, and Goettsch[35] found that roughly one-fifth of the body protein which was catabolized by the dog during a prolonged period of insufficient protein intake was withdrawn from the circulation, the remaining four-fifths being derived from the tissues outside the circulation. The latter authors observed that within the blood stream the relative contributions amounted to the following: hemoglobin, 18.7 per cent; albumin, 3.1 per cent; and globulin, 0.4 per cent. Sachar, Horvitz, and Elman[36] analyzed further the quantitative relationship between total tissue protein loss and serum albumin loss in dogs during a three-week period when maintained on a protein-free diet. These authors found that the serum albumin loss represented a small but relatively constant part (1 to 25 or 30) of the total protein lost from the body. In addition, when suitable protein was added to the diet, a similar relationship was found to hold for the increase of serum albumin and total tissue protein deposition. In other words, a loss (or a gain) of tissue protein and of serum albumin caused by diet paralleled each other, indicating the existence of a fixed partition between tissue protein and serum albumin.

Sachar and co-workers[36] pointed out that the latter conclusion applies only when the tissue proteins and serum albumin are altered as a result of diet and cannot be expected to hold in cases of hypoproteinemia due to blood loss, burns, nephrosis, or liver disease. They emphasize the practical clinical point, however, that in patients exhibiting hypoalbuminemia, primarily of nutritional origin, large amounts of protein and considerable periods of time may be required to correct the plasma and tissue protein deficiency.

From many studies it seems clear that the restoration of protein following a period of depletion from any cause must satisfy the demands of the tissues as well as the plasma. In problems of plasma protein replacement it is no longer permissible to think of the proteins of the plasma and of the tissues as entities. Indeed, evidence is continually mounting to support the views of Whipple and his colleagues,[14, 15, 20] that the plasma proteins are in a continuous and rapid exchange with the tissue proteins.

Maintenance of Nitrogen Balance by Intravenous Administration.—The literature pertaining to this subject has been critically reviewed recently by Elman.[37] It would appear that the evidence points definitely to the fact that nitrogen balance, at least for short periods, can be achieved both with plasma transfusions and with the intravenous injection of mixtures of amino acids either in crystalline form or as hydrolyzed protein. In fact, Whipple and Madden[15] have found that the dog can be maintained in a positive nitrogen balance, weight balance, and a state of health for weeks while receiving carbohydrate,

fat, minerals, and accessories by mouth and plasma protein as plasma by vein. There is some evidence[37] that compatibility is a prerequisite for maintenance of nitrogen balance with plasma transfusions, while the use of amino acid mixtures or of hydrolyzed protein preparations demands the presence of all of the essential amino acids for the maintenance of nitrogen balance.

There is evidence[38, 41] that better utilization occurs when plasma protein is given by vein than when given orally. On the other hand, with the use of amino acid preparations or hydrolyzed protein, utilization appears equally effective by either method of administration.[29, 30, 47, 48] That a large part of the injected protein leaves the circulation rather quickly and presumably enters the tissues has been observed by a number of workers.[40-46] This has been found to occur in normal as well as in hypoproteinemic states. It would appear, therefore, that the injected protein becomes distributed between the plasma and the tissues, possibly in proportion to their needs, which is in accord with the concept of a dynamic equilibrium between the plasma and tissue proteins.[14, 15] On the basis of these findings, it seems worth re-emphasizing that very large amounts of plasma (albumin) may be required to correct hypoalbuminemia in patients with chronic hypoproteinemia.

PLASMA PROTEIN AND FLUID EXCHANGE ACROSS THE CAPILLARY MEMBRANE

The exchanges between the blood and the interstitial fluids are confined almost entirely to the capillaries, which present an extremely large total area, are highly permeable to fluid, and are thus especially adapted for rapid transfers.[49] Further, the capillary wall appears to have the physical characteristics of an inert membrane (that is, nonsecretory), being permeable to water and crystalloids but relatively impermeable to the plasma colloids (chiefly protein). Since the blood in the capillaries is under a given hydrostatic pressure, it is necessary for the capillary membrane to be relatively impermeable to certain of the plasma constituents if plasma volume is to be maintained and, further, if there is to be a passage of fluid from the tissues into the blood against the pressure. The importance of the osmotic pressure of the plasma colloids in maintaining the equilibrium existing between blood and tissue fluid was first pointed out by Starling.[50] The concepts which are generally accepted at present recognize two principal forces (capillary blood pressure and plasma colloid osmotic pressure) and two secondary forces (tissue tension and interstitial fluid colloid osmotic pressure). The direction of fluid movement, therefore, will depend upon the relative magnitudes of the forces tending to drive fluid from the capillary (capillary blood pressure and interstitial fluid colloid osmotic pressure) and the forces tending to drive fluid back into the circulation (plasma colloid osmotic pressure and tissue tension). At the arteriolar end of the capillary the forces tending to drive fluid from the capillary are in excess of those tending to drive fluid into the capillary, while at the venous end of the capillary the reverse is true. Consequently, fluid leaves the capillary at the arteriolar end and is returned at the venous end. It is conceivable that at some point along the capillary the forces are in balance and at this point no fluid exchange would occur. By accepting the concept of a balance of forces, it is evident that a change in the magnitude of any one of the forces would affect the direction in which the fluid would flow. A familiar imbalance of forces is that due to a lowered plasma protein (that is, albumin) concentration, since with a fall of the plasma albumin the colloid osmotic pressure becomes reduced. Emphasis is

placed on the fall of albumin in this connection because it is a smaller molecule than globulin and exerts about 2.4 times the osmotic activity per gram.[51] With a fall of the plasma colloid osmotic pressure there would result a greater tendency to transudation and accumulation of interstitial fluid.

Epstein[56] was the first to point out the importance of a lowered plasma protein concentration in the pathogenesis of clinical edema. This fact has been widely confirmed subsequently and it has been shown[7] that when the total plasma protein concentration falls in adults to 5.5 ± 0.3 Gm. and albumin to 2.3 ± 0.2 Gm. (and in nephrotic children when the albumin falls to 1.2 Gm. per 100 c.c. or below[52]) edema is usually present. In other words, the osmotic pressure of proteins in normal human plasma amounts to about 30 cm. of water, and when the plasma protein (albumin) concentration falls, the plasma colloid osmotic pressure eventually becomes reduced to such a low level (about 20 cm. of water[53]) that usually the accumulation of interstitial fluid is sufficient to give evidence of palpable edema. Although there is some uncertainty regarding the specific osmotic pressure of the plasma protein fractions,[51] several authors have presented formulas by means of which the colloid osmotic pressure can be calculated from plasma protein concentration. Thus, Wells, Youmans, and Miller[54] found that their data could best be described by the equation, $P = C(21.4 + 5.9\ A)$, where P is the colloid osmotic pressure in millimeters of water, C is the total protein concentration, and A is the albumin concentration in grams per 100 c.c. of serum. Wies and Peters,[55] on the other hand, found the following equation to hold most accurately: $P = 60.1\ A + 22.9\ G - 50.0$, where P expresses the colloid osmotic pressure in millimeters of water and A and G represent albumin and globulin concentration in terms of grams per 100 Gm. of serum water. The water of serum may be estimated by the equation: Water = 98.40 – 0.718 Protein, where water and total serum protein are in terms of grams per 100 c.c.

The levels of the total serum protein and albumin concentrations referred to in the preceding paragraph are known as "critical levels." It is generally recognized, however, that the presence or absence of clinical edema in certain cases may not always be strictly related to the level of the serum protein concentration. Among the various factors[49, 57] which may alter the simple equilibrium conditions indicated previously, attention should be called to the basic assumption that the capillary wall is normally only very slightly permeable to protein. In this case the "effective" plasma colloid osmotic pressure would be practically equal to the true plasma colloid osmotic pressure. On the other hand, the escape of plasma protein into the interstitial fluid would reduce the "effective" plasma colloid osmotic pressure. It now seems reasonably clear that regional differences of capillary permeability exist and, further, capillary permeability may become altered; for example, as a consequence of circulatory disturbances or as a result of toxic damage to the capillary wall. As an additional factor, it is a common clinical experience that, in any condition where there is an imbalance of forces tending to increased transudation, the excessive accumulation of interstitial fluid will be exaggerated by the administration of sodium salts and water. In other words, an important factor in the accumulation of excessive amounts of interstitial fluid is the quantity of water and sodium chloride that is available.

It is evident that the latter factor must receive special attention when the intravenous administration of saline and/or glucose is contemplated in malnourished patients exhibiting dehydration. Not infrequently the serum protein

concentration at the time of first observation may be misleading and a false security may be placed in a normal concentration. If there has been a simultaneous loss of plasma volume, the loss of total circulating plasma protein may be obscured. Consequently, with a correction of dehydration by saline, intravenously, the serum protein concentration may fall below the "critical level" and edema may result. It is now generally appreciated that in such patients fluid replacement entails the use of plasma (or plasma substitutes) as well as saline.

SERUM ALBUMIN AND GLOBULIN CHANGES ENCOUNTERED CLINICALLY

Renal Disease.—That the serum protein concentration may be found altered in various types of Bright's disease is well established. In the nonhemorrhagic degenerative Bright's disease or nephrosis the serum protein concentration may be decreased markedly and the deficit is due almost entirely to albumin.[56, 58-60] In extreme cases the plasma albumin concentration may fall below 1 Gm. per 100 c.c., to be accompanied by marked edema. The tendency to edema formation in nephrosis is closely related to the serum protein (albumin) concentration and hence its determination is very important clinically. Recent attempts[41, 61, 62] to correct the hypoalbuminemia of nephrosis by injections of concentrated human blood serum or concentrated human serum albumin have yielded results which are not too encouraging, but further careful investigations seem warranted.

Hemorrhagic nephritis or glomerulonephritis may be accompanied by serum protein deficits which approach those observed in severe nephrosis. In acute glomerulonephritis the serum protein concentration may remain within normal limits throughout the illness if the course is mild.[60, 63] Such cases may exhibit rather marked edema despite the fact that the serum protein concentration is within the normal range of variation. This represents an outstanding example of the lack of relationship between the serum protein level and tendency to edema, and it has been generally held that in acute glomerulonephritis the capillaries are probably injured and abnormally permeable to protein, thereby increasing the tendency to edema. However, recent observations by Warren and Stead[64] have left some doubt that this is true. The latter authors examined the protein content of edema fluid collected from the subcutaneous tissues of the leg and from the sacral region of patients with acute glomerulonephritis. It was found that the values for the total protein content, which varied between 0.1 and 1.0 Gm. per 100 c.c., did not differ significantly from those obtained on the edema fluid of patients with congestive heart failure. The authors expressed the view that, with the evidence at hand, the cause of edema in acute nephritis is water and salt retention secondary to a disturbance in renal function and not the result of diffuse capillary damage throughout the body. In any event, unless a serum protein deficit ensues, the edema is usually only of short duration. In the more severe cases and as the condition is prolonged a serum protein deficit occurs wherein the albumin concentration may fall to a level definitely below 2 Gm. per 100 c.c.[58, 60, 63] In these patients edema is invariably present when the albumin concentration is below 2.2 Gm. per 100 c.c.,[63] and the tendency toward edema remains until the serum protein rises above the critical level.

Chronic glomerulonephritis, the nephrotic type, or chronic active hemorrhagic nephritis is accompanied by a decrease in the serum protein concentration, representing chiefly a deficit of albumin.[59, 60] The presence or absence of edema in this condition appears directly dependent upon the serum protein

level. As the condition progresses from the acute or the chronic stages to terminal glomerulonephritis, there is a tendency for the serum protein concentration to increase to normal. This apparently happens in about 50 per cent of the cases,[60] although lowered serum protein concentrations are not infrequently encountered.[65]

Liver Disease.—The alterations of the serum proteins in hepatic disease have attracted the attention of many workers and the change most frequently encountered (particularly in cirrhosis of the liver) is a deficit of the albumin fraction.[8, 66, 71] The globulin concentration is generally elevated so that in many patients the total serum protein concentration is within the normal range of variation. Not infrequently, however, the deficits of albumin are greater than the increases of globulin so that the total serum protein concentration is lowered. Although malnutrition may be a factor contributing to the hypoalbuminemia encountered,[8] various workers[67, 70, 71] have emphasized the feature of an impairment in the fabrication of albumin.

Malnutrition.—The importance of malnutrition (inadequaate protein intake and/or improper assimilation) as a predisposing factor toward the hypoproteinemia observed in certain diseased conditions has become generally recognized.[8, 72-74] The hypoproteinemia of malnutrition is due to a deficit of the albumin fraction; the globulin (except in cases complicated by infection) remains relatively constant. As was pointed out, experiments on dogs have revealed the fact that tissue protein as well as serum protein becomes depleted when the protein intake is inadequate. With a fall of the serum albumin, the plasma volume also becomes reduced, and this frequently obscures the magnitude of total circulating plasma albumin loss.

Plasma Protein Deficiency in Trauma.—A fundamental problem in trauma (including hemorrhage, burns, "crush-injuries," peritonitis, and intestinal obstruction) is the correction or prevention of plasma (protein) depletion.

Hemorrhage: It is now clear that an understanding of the pathologic physiology resulting from acute blood loss is of great importance in the management of many medical and surgical patients. In the past, attention of the clinician has been directed principally to the restoration of blood cells and hemoglobin following acute hemorrhage. However, recent studies indicate that restoration of plasma volume and total circulating plasma proteins are equally important phenomena since these processes help to re-establish the hemodynamics of the peripheral circulation so that the hemoglobin remaining in the circulation can carry out its function of transporting oxygen to the tissues.

From studies which have been carried out on normal dogs[75-82] and on human subjects[83, 84] it would appear that following a single non-fatal hemorrhage there is a prompt, but gradual, inflow of fluid and protein into the circulation. The initial increase of plasma volume is apparently the result of the addition of fluid relatively low in protein, and in from several hours to some seventy hours the plasma volume may exceed the control level. In general, it has been found that the plasma protein (albumin) is restored more slowly than the plasma volume and may still be subnormal at the end of several days. Apparently the slow return of plasma volume cannot be attributed to a lack of fluid in the body with which to dilute the blood, since physiologic saline, when given intravenously, does not maintain blood dilution but leaves the circulation rapidly.[77, 84] Likewise, the even slower return of the plasma albumin has been taken as evidence against the existence of a reserve of preformed plasma protein which

can enter the blood stream in the first few minutes after hemorrhage.[80] It seems more likely that the protein entering the circulation under these circumstances represents protein stored as a part of tissue protein outside the circulation or that protein building materials are readily available for the fabrication of new protein. Since considerable evidence points to the view that there is a continuous traffic of plasma protein leaving and entering the circulation and that there is a continuous exchange between the plasma proteins and the tissue protein stores, the initial increase of total circulating plasma albumin following hemorrhage may be a redistribution of mobile plasma protein. It is of some interest in this connection that dogs made hypoproteinemic by diet are capable, without intravenous fluids, of restoring the plasma volume to the prehemorrhage level and of adding a small amount of plasma albumin to the circulation following a single nonfatal hemorrhage.[85]

From experiments in which shock has been produced by graded hemorrhage[80, 86] there appears to be a deficiency in the ability of the organism to dilute the blood and to add plasma protein to the circulation. In fact, as evidenced by a rise of the hematocrit, there may be an actual loss of plasma fluid and plasma protein from the circulation when shock develops.[86] On the other hand, there is evidence[87-89] against the existence of an increased permeability of the general capillary bed to plasma protein in hemorrhagic shock. The progressive decline of the plasma volume in hemorrhagic shock might well be attributed to a progressive fall in the volume of actively circulating plasma associated with peripheral stagnation.[87-89]

The intravenous injection of physiologic solution of saline may be effective in restoring the blood pressure following mild hemorrhage. It is quite ineffective, however, in patients with surgical shock and profuse hemorrhage. Even following nonfatal hemorrhage the injected physiologic solution of saline rapidly leaves the circulation[77] and in shock it may actually aggravate the escape of plasma protein from the circulation.[89]

Burns: Following severe burns there is usually a marked negative nitrogen balance[90-92] which may persist for days. In addition to an increased urinary nitrogen output, the nitrogen loss in the exudate from surface burns may represent a sizable per cent of the total nitrogen lost from the body.[92] An outstanding feature of burn shock is the extreme degree of hemoconcentration associated with a diminished plasma volume, attributable to a leakage of plasma from the capillaries in the burned area and in the tissue surrounding it. Increased capillary permeability appears to exist only in the regions near the burn and apparently does not include the capillaries in an area remote from the burn.[88, 89, 93, 94] The serum protein concentration may show little change but is generally reduced. On the other hand, the total amount of circulating plasma albumin is always reduced in severe burns.

Crush Injuries: The release of compression, due to fallen beams, masonry, or debris pressing heavily upon some part of the body, usually an extremity, has been found to be followed in a few days by oliguria which in certain cases progresses to anuria and death.[95, 96] Neither the duration of the compression nor the amount of the body involved appears to be directly proportional to the ultimate outcome.

It has become evident that the injury results in degeneration and necrosis of the muscle. Apparently as the result of tissue trauma there is an altered permeability of the capillaries and plasmalike fluid escapes into the injured area. The loss of plasma volume leads to hemoconcentration and fall of blood

pressure. The serum proteins may be increased at first and then return to normal. In some cases the serum proteins become reduced, particularly following the administration of crystalloid solutions, and in the latter instance there may be widespread edema.

Although the circulatory effects of crush injuries are understandable, the more important sequela of this type of trauma, renal failure ("crushed syndrome"), is more difficult to interpret. When oliguria occurs, the urine is brownish in color and complete suppression of urine ultimately supervenes. Apparently various products from the necrotic muscle [myohemoglobin (myohematin), potassium, phosphate, and possibly other substances] escape into the circulation when it is re-established locally following the release of compression. The release of such products may contribute to the severe renal impairment with death in uremia which follows in about 66 per cent of the patients.[95, 96]

Peritonitis and Intestinal Obstruction: Clinical and experimental studies[97-99] have demonstrated that in spreading peritonitis and intestinal obstruction hypoproteinemia affecting primarily the albumin fraction is of frequent occurrence. In both of these conditions there is a loss of fluid containing protein in the walls of the intestines and the peritoneal cavity. There is, therefore, a decrease of plasma volume which may be detected by an increase of the hematocrit. In many ways the pathogenesis is similar to that presented by severe burns.

Hyperproteinemia: A number of years ago Wu[100] noted that in kala-azar the total serum protein concentration may be increased to 9 to 10 Gm. per 100 c.c. The increase was found to be due to a rise of the globulin fraction, the albumin, on the other hand, being definitely reduced. With subsequent accumulation of data it seems permissible to draw the generalization that whereas hypoproteinemia is due chiefly to a deficiency of albumin, hyperproteinemia is due to an increase of the globulin fraction. Kagan,[17, 18] reporting observations carried out on a large series of cases, with special attention to the incidence of hyperproteinemia, concluded that hyperglobulinemia appears principally in diseases involving the bone marrow, chronic infections, diseases involving the liver, and in dehydration. A number of authors have reported the serum globulin to be increased significantly in patients with multiple myeloma,[6, 18, 101-103] chronic infections of various origins,[6, 8, 18, 75] lymphogranuloma inguinale,[103, 104] Boeck's sarcoid,[103, 105] and cirrhosis of the liver.[6, 104] In acute dehydration the serum protein concentration becomes increased, both the albumin and globulin fractions being affected. Not infrequently, however, the increased serum protein level in dehydration is due to increased globulin, the albumin concentration being within the normal range of variation or even reduced.[18]

Although further work appears desirable, recent electrophoretic studies of sera from patients exhibiting hyperproteinemia are of interest. Attempts to correlate the serum electrophoresis findings with protein concentrations as determined by the salting-out technique of Howe[1] have raised doubt that the fractionation of serum based upon solubility in salt solutions represents a clear-cut division. Thus, in normal human serum[6] the electrophoretic pattern was found to be essentially unchanged after precipitation in 13.5 per cent sodium sulfate (after the removal of euglobulin). About one-half of the gamma, one-fourth of the beta, and none of the alpha components were found to be removed by precipitation in 17.4 per cent sodium sulfate (after euglobulin and pseudoglobulin I had been removed. All of the gamma, about three-fourths of the beta, and one-fourth of the alpha components were found to be removed by precipitation in 21.5 per cent sodium sulfate (after everything except the albumin had

supposedly been removed). In other words, a considerable amount of the α- and β-globulins were found to have remained with the albumin. Longsworth, Shedlovsky, and MacInnes[3] observed the electrophoretic patterns of sera from patients showing a variety of pathologic conditions, with fever in common, and all showed an increased α-globulin content. In patients with portal cirrhosis the β- and γ-globulins were found to be increased[5] and the γ-globulin strikingly so when ascites was present. Gutman and co-workers[6] carried out serum protein studies by the Howe method in thirty-eight patients with multiple myeloma and electrophoretic analyses in ten patients. As the result of these studies the authors concluded that most myelomatous sera fall into one of the following three classifications: (1) sera with hyperglobulinemia due to an increase chiefly in the Howe euglobulin fraction, partly to an increase of the pseudoglobulin I fraction and, exhibiting electrophoretically an increase in the gamma components; (2) sera with or without hyperglobulinemia giving a variety of anomalous patterns by the Howe or electrophoretic methods, for the most part due to Bence-Jones proteinemia; and, (3) sera of apparently normal composition with respect to serum proteins.

SUMMARY

From evidence which is now available it would appear that the normal organism has a marked capacity to synthesize plasma proteins under conditions of stress. The "building blocks" for plasma protein fabrication may be supplied as free amino acids, hydrolyzed protein, or plasma either directly by vein or orally. For maintenance of nitrogen balance, however, it is necessary to supply in optimal amounts all of the essential amino acids. Infection and intoxication appear to retard plasma protein regeneration.

There is much to support the view that the plasma proteins are in a continuous traffic, leaving and entering the circulation and undergoing a continuous exchange with the tissue proteins. During periods of restricted or inadequate protein intake, the excess nitrogen lost from the body is contributed both by the plasma proteins and tissue proteins; the ratio of the loss of plasma albumin to tissue protein is approximately 1 to 25 or 30. With the resumption of an adequate protein intake, plasma albumin and tissue protein are restored in approximately the same ratio. This emphasizes the practical point that in patients with serious protein depletion, primarily due to malnutrition, the correction to normal may require the administration of large amounts of protein and a considerable period of time may be necessary.

Under normal conditions the escape of plasma protein (albumin) from the circulation is sufficiently restricted so that it can carry out its important function of sustaining the plasma volume. In "shocklike" conditions (including burns, crushing injuries, peritonitis, and possibly severe hemorrhage) the integrity of the membrane becomes disturbed. This results in a loss of protein and fluid from the plasma, which may lead to serious consequences.

Significant disturbances of the serum protein concentration may be frequently encountered clinically. It seems permissible to draw the generalization that whereas hypoproteinemia can be attributed chiefly to a deficiency of albumin, hyperproteinemia is due to an increase of the globulin fraction. Hypoproteinemia may be encountered, for example, in Bright's disease, malnutrition associated with various conditions, liver disease, and various conditions associated with plasma protein loss. It would appear that hyperproteinemia is not

so rare as was once believed. Hyperproteinemia (hyperglobulinemia) may be encountered principally in diseases involving the bone marrow, chronic infections, diseases involving the liver, and in dehydration.

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LABORATORY METHODS

MODIFICATION OF THE HAMILTON OPTICAL MANOMETER

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THE metallic membrane manometer, originally designed by Hamilton, Brewer, and Brotman,[1] was, in several respects, unsatisfactory for the work to be done in the Research Section of the School of Aviation Medicine:

1. Adjustment by eight set screws was time consuming and the suspension from a horizontal bar rendered the instrument vulnerable to extraneous vibrations.

2. Sensitivity was so limited that the long optical arm made operation of the instrument in a limited space complicated and awkward.

3. The assembly was composed of three units: a source of light, a camera, and a bank of manometers, each individually mounted and somewhat difficult to align.

4. The source of light consisted of a 500-watt, monoplane, filament projection lamp enclosed in a ten-inch, cubical brass box with an adjustable slit for casting the beam of light on the mirrors. This arrangement was found to be unsatisfactory because of the excessive heat generated and because of the leakage of light from vents in the box. Incorporation of a fan and motor blower was unsatisfactory because of the vibration created.

5. Finally, cameras for 12 cm. bromide paper are difficult to purchase and quite expensive. The one constructed in this laboratory is simple, easily assembled, and entirely satisfactory.

The electrical capacitance diaphragm manometer described by Lilly[2] was considered for use, but the need for multiple simultaneous records and therefore multiple pieces of this apparatus nullified one important requirement of the instrument for the research to be done; namely, compactness.

DESCRIPTION OF APPARATUS

The Assembly.—A table for the manometers measuring 30 by 30 inches was so constructed that it could be immobilized on tripod supports or moved about on casters. To prevent parallax and to facilitate focusing, the height of the table is adjustable in order to bring the mirrors on a level with the slit of the camera. Although the size of the table may appear to be excessive, it was found that the space on it in front of the manometers is convenient for holding antiseptics, sponges, and other necessary items for an experiment. The base of each manometer carrier was attached to a tripod pan and tilt head (Fig. 1). These in turn were secured to a cast iron bar, 1¾ inches high, at one side of the table top. A manometer may be quickly aimed at the camera and fixed in position by simple manipulation of the adjustment handle of the pan and tilt

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head. The housing, clamped within the carrier, is a circular brass tube with eight set screws supporting the manometer.

Frequency and Sensitivity of Apparatus.—Sensitivity may be be augmented in three ways: by increasing the distance of the optical arm (distance from light source and camera to the manometer), by decreasing the thickness or type of membrane, and by increasing the diameter of the manometric system and hence the cross-sectional area through which a force may act upon the membrane. It is impractical to lengthen the optical arm, particularly if the instrument is to be employed where conservation of space is an important item. Membranes with a thickness of 0.001 inch or less tend to buckle when exposed to arterial pressures. Enlarging the diameter of the system increases the effective mass and decreases frequency. Hamilton, therefore, set the optimal optical

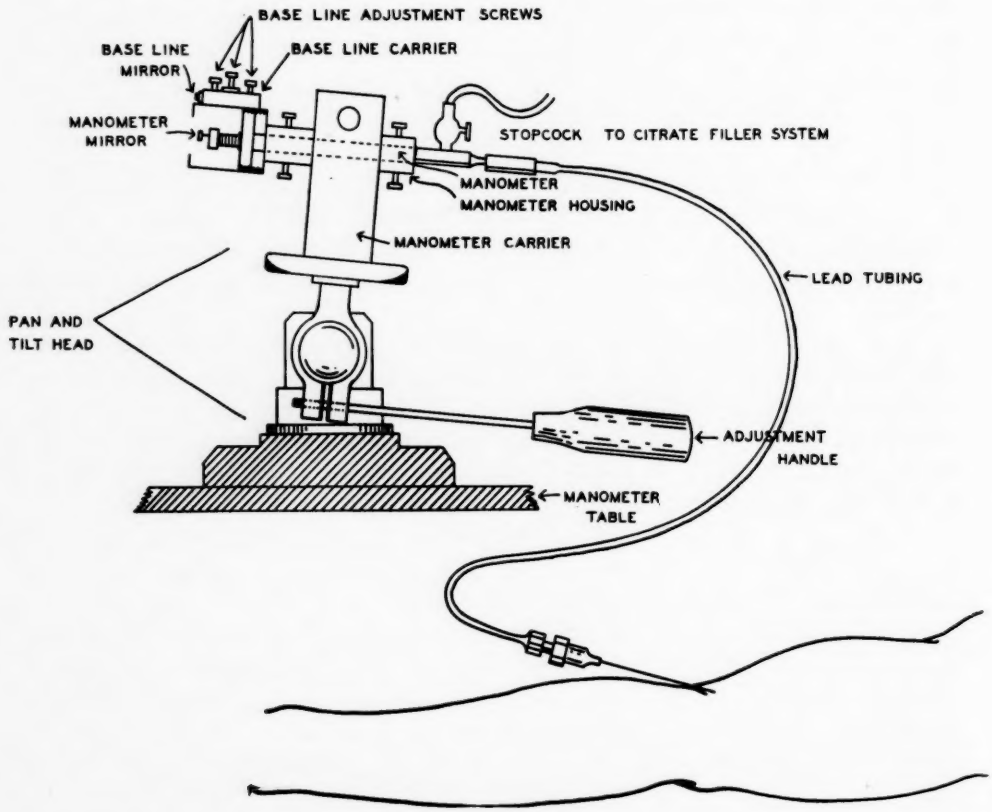

Fig. 1.—Manometer assembly (side view).

distance at two meters and the most favorable mass of a system yielding adequate natural frequencies for membranes in excess of 0.001 inch in thickness as follows: manometer, $\frac{3}{16}$ inch in diameter, $4\frac{3}{8}$ inches in length; lead tube, approximately 42 inches in length, $\frac{1}{8}$ inch in diameter; 18- or 20-gauge needles. Employment of the instrument in small spaces, however, demands greater compactness, shorter optical distances, and hence less sensitivity. To counteract this, it seemed plausible to increase the diameter of the manometer. Accordingly, manometers were constructed with bores $\frac{5}{16}$ inch in diameter. Natural frequencies remained satisfactory and sensitivity was usually increased twofold for identical membrane thicknesses. The larger caliber manometers so enhanced sensitivity, and consequently analysis of records, that we now employ them for

all recordings. An optical lever of one meter may be used, but where greater space permits, this lever is increased to two meters.

In order to record arterial pressures accurately, it is necessary to have manometric frequencies in excess of 100 c.p.s.[1] Frequencies are determined when each manometer is assembled and checked at short intervals thereafter. The system is filled with sterile citrate solution and suction is applied at the open end of the needle. Suddenly releasing the pressure throws the manometer into free vibrations of its own natural frequency which may then be photographed.

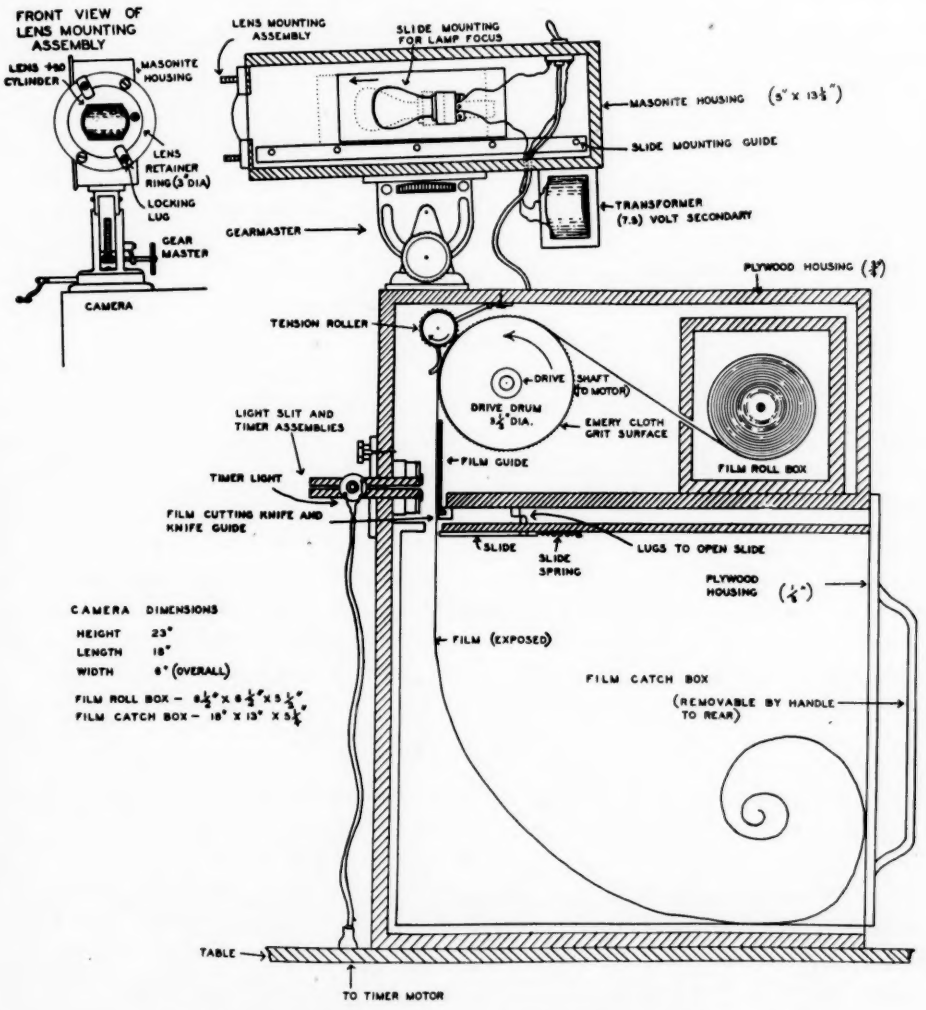

Fig. 2.—Light source and camera assemblies (left side).

Calibration.—Immediately upon completion of an experimental procedure, all manometers are calibrated against the mercury column. It is important that the needles remain in a position identical to that when the record was taken. They are plunged into a thick rubber tube, one end of which is clamped and the other connected to the mercury calibrating manometer, and a series of records taken as the mercury is elevated by successive increments (Fig. 4).

Membranes.—Many metals have been used to construct membranes. We have successfully employed both coin silver and beryllium copper in thicknesses

ranging from 0.0005 to 0.002 inch. The beryllium copper must be heat treated in a sand bath at 500° F. for one hour to assure proper "springiness." The membrane is cut to a diameter just exceeding that of the manometer. A tiny stud is eccentrically soldered to its outer surface before it is transfixed to the manometer by the cap nut. The mirrors, either front or rear surface, are cut from 0.5 D planoconvex lenses, measure approximately 4 by 4 mm., and focus sharp vertical beams of light at two meters. Ordinary rubber cement has proved satisfactory for mounting them to the membrane stud.

Light Source.—On top of the camera is the light box (Fig. 2) constructed of masonite ⅛ inch thick. It rests on a Bolex gearmaster allowing aiming of the beam of light in four directions. Once adjusted, locking lugs on the gearmaster prevent accidental movement of the light box. A 50 c.p. automobile headlight bulb, with its filament in the vertical position, is attached to a slide within the box which, in turn, may be moved back and forth by means of a small lever extending through a slit to the outside. Slide adjustment is necessary to focus the beam of light through the +9.0 D cylinder at the front of the light box. The cylinder, mounted in a circular retainer ring, may be rotated upon loosening two locking lugs. Its axis must be horizontal for the reflected beam to cast a horizontal rectangle of light on the manometer bank. Momentary adjustment of the bulb slide, gearmaster, and lens retainer ring is usually necessary preceding each experiment.

The masonite light box housing, together with the small bulb, prevents excessive heating of the unit and renders ventilation (and coincidental light leakage) unnecessary. Adjustment of the light beam by trial and error is simple, requiring only a few moments to obtain sharp vertical lines from the mirrors. Finally, the light source and camera have been incorporated on a single mount, facilitating compactness, mobility, and ease of alignment.

Camera.—The camera (Fig. 2) is housed in plywood ¾ inch thick and is composed primarily of two parts: upper (load) and lower (film catcher) boxes. Access to the load box is through a slide panel, removable to the rear. It accommodates 175 feet of 12 cm. bromide paper which is inserted on a roll after removal of a plywood shield transfixed to the box by a wing screw. The film is brought through a slit in the front of the load box and thence over a drive drum covered with a grit-surface emery cloth. Purchase of film to the drum is aided further by a tension roller suspended (without springs) from the roof of the camera to rest upon the drive drum. A vertical strip of spring steel guides the film past the exposure slit into the film catcher box. Exposed film is cut with a knife mounted in a guide just above the film catcher box and operated by a lever attached to the side of the camera (Fig. 3). When the catcher and its exposed film is removed, further exposure is prevented by a slide and spring arrangement which closes the slit through which the film entered the box.

A diagram of the light slit in the camera may be seen in Fig. 2. The entire assembly, together with the timer light, is constructed as a single unit attached to the face of the camera by a transfixion screw above. The width of the slit just in front of the recording paper is approximately 1/64 inch. The tunnel in front of it prevents dispersion of light among the several beams and reduces the parallax.

The timer light (Fig. 3) is constructed to cast a point of light on the side of the film each second (Fig. 4, lowest record). A telechron motor beneath the camera table, operating from a 115-volt A.C. primary circuit, describes 1 r.p.s. It is equipped with a contact arm activating, through a transformer, a small

bulb at one end of the light slit. A tiny mirror reflects this light through a 27-gauge hypodermic needle to the film.

The motor, mounted on the side of the camera, is a small Holtzer-Cabot type operating from a 115-volt A.C. circuit through 60 cycles. It is so geared to the drive drum that camera speeds of 2 and 6 mm. per second are available. The faster speed is adequate for recording contour variations in arterial pressure, whereas the slower speed is more convenient for analyzing (in absolute systolic and diastolic pressures) slow changes over longer periods of time.

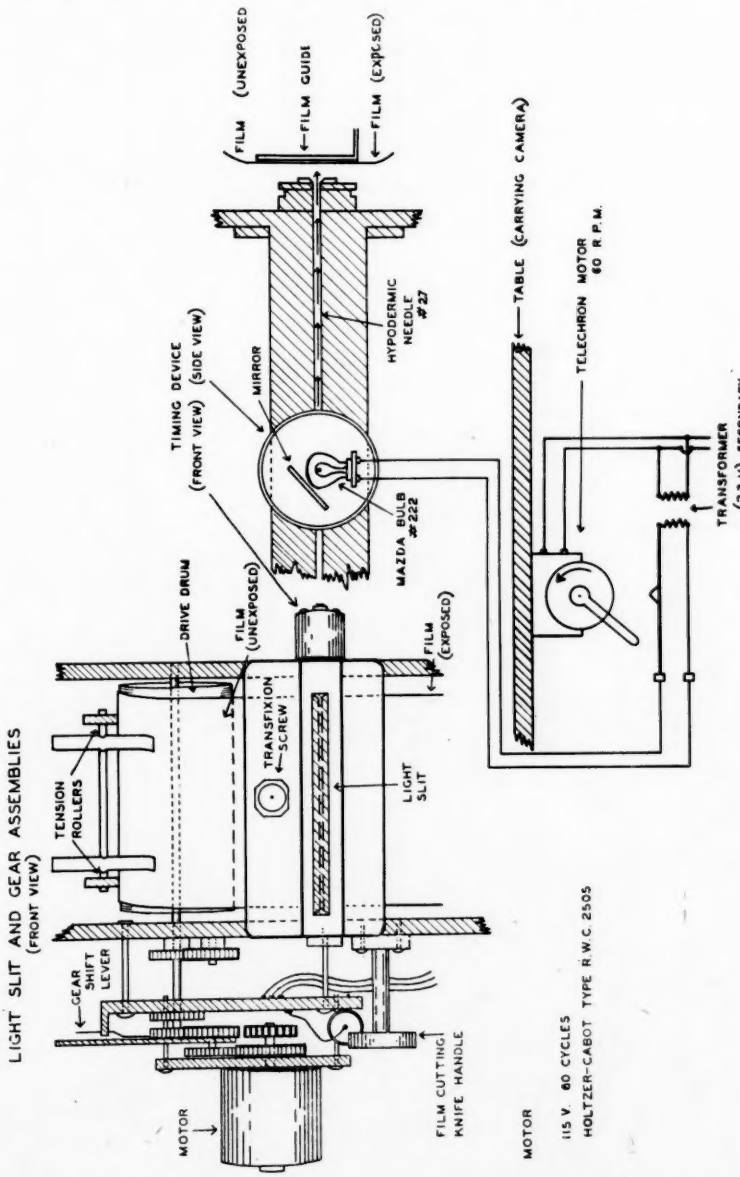

Fig. 3.—Details of gear assemblies in camera and of timing device.

Records.—In Fig. 4 is a simultaneous record of the intrapulmonic pressure, the arterial pressure, and the venous pressure during the performance of a Valsalva experiment in man after a deep inspiration (called "Vi" maneuver). For the intrapulmonic pressure an air-filled system was used; for the other pressures a fluid-filled system was used. Thicknesses of the membranes used depended on the sensitivity desired. In this record, taken at an optical distance of

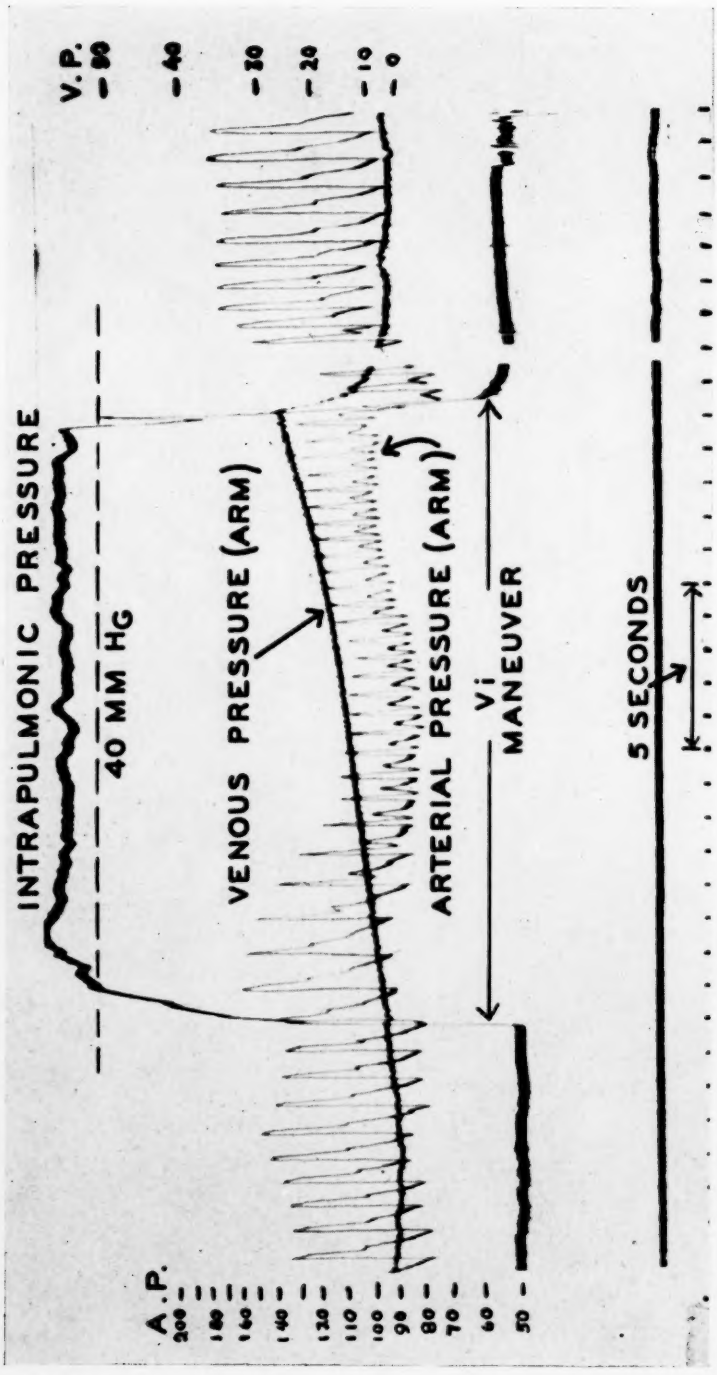

Fig. 4.—Simultaneous record of the intrapulmonic pressure, the brachial arterial pressure, and the antecubital venous pressure recorded with the modified optical manometer during the performance of a Valsalva maneuver by a human subject after a deep inspiration (Vi maneuver). *A.P.*, Arterial pressure; *V.P.*, venous pressure.

two meters, the membranes were of the following thicknesses: arterial pressure, 0.0015 inch; venous pressure, 0.00075 inch; intrapulmonic pressure, rubber dam.

CONCLUSIONS

1. The Hamilton manometer was modified for use in limited spaces for recording arterial and venous pressures particularly.

2. Changes were made in the mounting, the bore, and the method of calibration of the manometer and in the camera and source of light.

The authors are grateful to Mr. Frank V. Garbich, who did the construction; Lt. S. Rodbard, for suggesting the use of the pan and tilt head on the manometer; Dr. W. F. Hamilton, of Augusta, Ga., and Dr. L. N. Katz, of Chicago, for suggestions and essential parts; Technician 4th Grade Walter Wagner, for technical assistance; and Sgt. Ted Wade, for the sketches.

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A GUINEA PIG HOLDER FOR JUGULAR AND CARDIAC NEEDLING

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A BIVALVED plaster cast has been found much more convenient than the conventional animal board for jugular and cardiac needling. Animals are immobilized more quickly and more thoroughly. Much time is saved and fewer

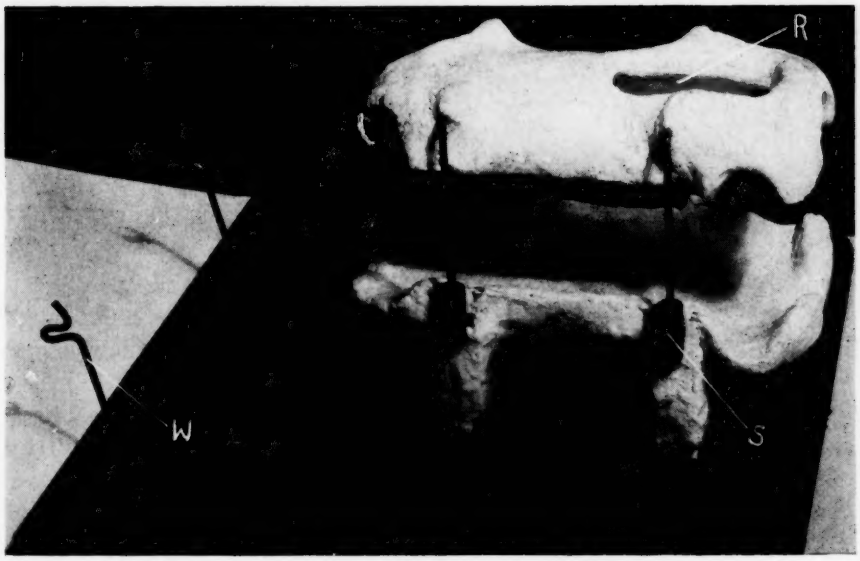

Fig. 1.—*R*, Respiratory vent; *S*, setscrew; *W*, wire hook.

From the Allergy Clinic, Department of Internal Medicine, University of Virginia Medical School.

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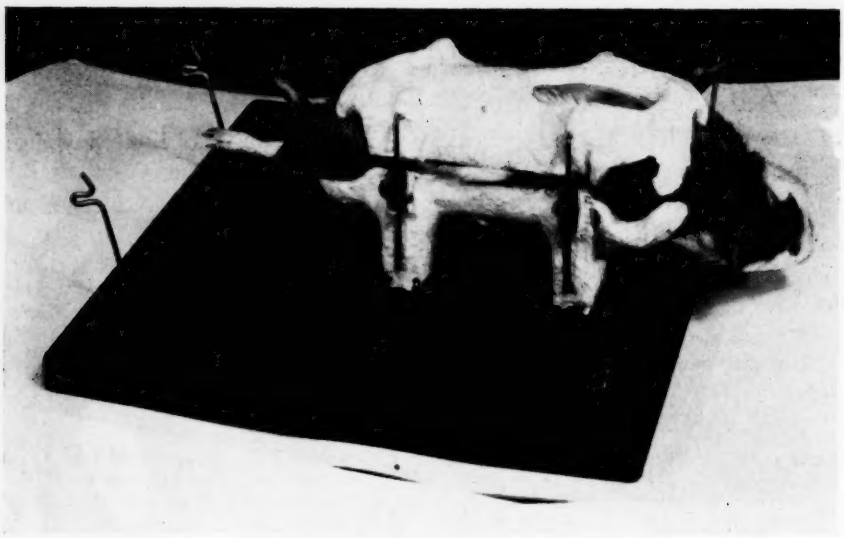

Fig. 2.

veins are torn by struggling during injections or aspirations. The cast is cut out so that it is not soiled by urine or feces.

The essential details of the holder are illustrated in Figs. 1 and 2. Variation in size of the pigs is accommodated for by the opening at the posterior end and by separation of the two halves. With small pigs a snug fit is obtained by placing a cloth pad beneath them. The wire hooks are used to immobilize the legs for femoral and saphenous punctures. The hooks are not necessary for jugular or cardiac needling.

PRECIPITIN REACTION: QUANTITATIVE MEASUREMENT WITH A MERCURY SEALED CAPILLARY "J" TUBE

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Charlottesville, Va.

JOHNSON and associates[1] described a modification of the Meloche microcentrifuge tube[2] used in the quantitative measurement of precipitin reactions. They used paraffin and a broad rubber band over the ends instead of sealing the capillary tube in a globule of mercury as was done by Meloche. They also reviewed the literature on this subject.

This report describes another modification of the tube which retains all the advantages previously recognized and greatly simplifies the method of sealing and cleaning.

The capillary section of the tube is made about 5 cm. longer than usual to permit bending in the form of a "J." A small filling bulb is put in the end. A drop of mercury put in this bulb effectively seals the lower bend of the "J."

From the Allergy Clinic, Department of Internal Medicine, University of Virginia Medical School.

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The antigen-antibody mixture is placed in the large end of the tube. Adequate centrifugation packs the precipitate down on the mercury. The apparent volume of precipitate can be measured quantitatively with the tube in the vertical position (height $\times \pi r^2$).

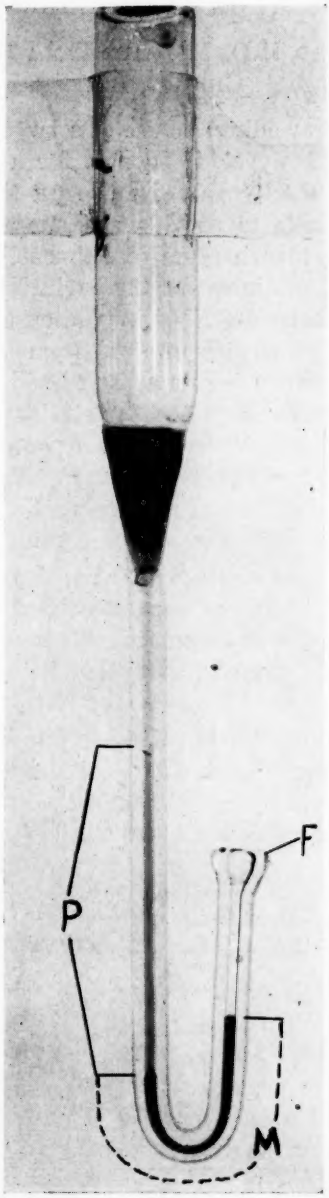

Fig. 1.—*P*, Precipitate; *M*, mercury; *F*, filling bulb.

To clean the tube simply empty the mercury and suck cleaning fluid through it.

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A POLYVALENT SERUM FOR THE DIAGNOSIS OF SALMONELLA

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THERE is no means of avoiding the use of a large set of sera for the typing of Salmonella. It is, however, impossible for a small laboratory to carry such a number of sera in stock. In order to meet the requirements of smaller establishments, it is desirable to provide a serum which will give a positive reaction with most of the known types of Salmonella. Such polyvalent diagnostic serum containing agglutinins for the agglutinogens of Salmonella found in the United States has been described by Galton and Quan.[1] Experience of the authors with Salmonella strains received from the tropics and subtropical regions called their attention to the necessity of widening the range of activity of the polyvalent sera to include less common strains.

A new polyvalent diagnostic serum was devised therefore which contains antibodies for one or more of the antigenic factors of all known Salmonella strains, with the exception of three rare types: *Salmonella Cerro, Salmonella Arizona,* and *Salmonella Ballerup.* The serum is prepared as follows:

1. Motile cultures of Salmonella, checked with the aid of some appropriate method, for example, that of Hajna,[2] are inoculated separately into nine tubes containing measured amounts of 1 per cent sterile proteose-peptone broth with ½ per cent sodium chloride, pH 7.2 to 7.4. The Salmonella listed in Table I are used.

After twenty-four hours of incubation at 36 to 37° C., 0.85 per cent saline containing 0.6 per cent formalin is added to the tubes. The amount of the formol-saline should be equal to the volume of the broth in the tubes. The contents of the tubes are poured together into a sterile flask and preserved in the icebox.

TABLE I

SALMONELLA	ANTIGENIC STRUCTURE		
S. paratyphi A	I, ¶I, XII	a	
S. schottmuelleri (Variety java)	IV, V, XII	b	
S. typhimurium	IV, XII	i	1, 2, 3 . . . . .
S. Hartford	VI, VII	y	e, n, z_{16} . . .
S. Muenchen	VI, VIII	d, d_3, d_4	1, 2 . . . . .
S. enteritidis	I, IX, XII	g, o, m, z_1, z_2	
S. London	III, X, XXVI	l, v	1, 4, 6 . . . . .
S. Selandia	III, XV	e, h	1, 7 . . . . .
S. Senftenberg	I, III, XIX	g, s, t, z_8, z_9	

At least two rabbits are inoculated into the ear vein with 0.5, 1.0, 2.0, and 2.0 c.c. of this mixed "H" antigen every fourth day.

2. The surface of eight nutrient agar slants, containing 1 per cent proteose-peptone, ½ per cent sodium chloride, and 1.5 per cent agar, pH 7 to 7.2, are inoculated heavily with the Salmonella strains listed in Table I, with the exception of *Salmonella typhimurium.* After twenty-four hours of incubation at 36 to 37° C., the surface growth is washed off with small amounts of absolute ethyl alcohol and the washings from all tubes collected into one flask and mixed

From the Enteric Center of the Chicago Medical School.
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and distributed into centrifuge tubes. The tubes are kept for one hour at 56° C. in the water bath, are centrifuged, and the alcohol drained off and substituted with equal volumes of 0.85 per cent saline containing 0.3 per cent formalin. This mixed "O" antigen may be preserved in the icebox for several weeks.

The rabbits treated with the mixed "H" antigen receive 0.5, 1.0, and 2.0 c.c. of this antigen every fourth day.

3. Finally, the rabbits are given intravenous injections of 0.5, 1.0, and 1.0 c.c. of a mixture of live, twenty-four-hour-old proteose-peptone broth cultures of all Salmonella strains listed in Table I. After test bleeding, the serum is harvested and preserved by the addition of an equal volume of glycerin. It is used both for slide and for tube agglutination tests, using the known and reliable technique of Edwards and Bruner.[3]

The serum described gave positive results with 97 stock cultures and 276 freshly isolated strains belonging to the groups "A" to "E" of Salmonella in dilutions 1:3 and higher, when the slide method was used. It agglutinated 110 stock cultures and 293 freshly isolated Salmonella strains, members of the groups "A" to "F," in dilutions 1:160 and higher, when the test tube method was applied. Two strains of *S. Ballerup* and one strain of *S. Cerro* were not agglutinated by this serum.

It is believed that this polyvalent serum may aid in the simplification of enteric work both in laboratories handling specimens from the United States and from abroad.

SUMMARY

The preparation of a polyvalent diagnostic serum for Salmonella is given. This serum may be used for slide agglutination tests of Salmonella belonging to the groups "A" to "E." It gives positive test tube agglutination reactions with Salmonella which are members of these groups and with most strains belonging to group "F," i.e., with about 99 per cent of all known Salmonella types.

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THE USE OF TEST PAPER FOR THE RAPID ESTIMATION OF SULFONAMIDES IN BLOOD AND OTHER BODY FLUIDS

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SANITARY CORPS, UNITED STATES ARMY

A SIMPLE and rapid method for estimating the level of sulfonamide in blood is of much clinical value. The importance of knowing the concentration of free drug circulating in the blood stream of sulfonamide-treated patients is unquestioned. Colebrook[1] has pointed out that a therapeutic sulfonamide level is desirable and more effective at the onset of a bacterial infection and that the action of the drug is favored by fever temperatures.

From the Laboratory Service, Battery General Hospital, Rome, Ga.
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Quick methods for estimating sulfonamides should retain the accuracy of the older methods, reduce the number of steps used, and, if possible, minimize the inconvenience to the patients. In a previous communication[2] I reported a rapid method using a test paper for estimating the concentration of sulfonamides in serum. The method differed radically from the usual procedures, and the preliminary results obtained indicated several points of superiority over tests in current use. The object of the present paper is to present data obtained in the actual clinical application of the method in an Army General Hospital. Results are reported on 518 samples of blood from patients being treated for a variety of diseases in which sulfonamide values were obtained by two methods. In each case the test paper value was used as a check on a parallel test run with the procedure of Bratton and Marshall.[3] Incidentally, by predicting the sulfonamide level of the unknown with the test paper, it was possible to set up only one standard of a value in close proximity to that of the unknown when using the Bratton and Marshall method.

In my previous communication two questions were raised: (1) whether the test paper maintains its integrity over a period of time and (2) what effect varying concentrations of proteins in serum, as well as alterations of proteins in pathologic conditions, might have on the color produced, which might lead to inaccuracies. We are now ready to report more fully on these questions. Because the method has proved of real value in this hospital, a more detailed description of the procedure will be given.

The test makes use of Ehrlich's reagent, p-dimethylamino benzaldehyde, in the formation of a Schiff base with primary aromatic amines, as first described by Kuhnan in 1938.[4] The test paper contains this reagent with a sufficient quantity of oxalic acid to give a pH of 2.5 when wet with serum or other biologic fluid. By the use of the test paper, the level of sulfonamide can be estimated directly in plasma, serum, and other clear biologic fluids, without the addition of precipitating or acidifying agents. The orange-yellow color produced is evaluated by comparison with an appropriate color chart or with colors produced simultaneously from solutions of known concentrations.

PREPARATION OF TEST PAPER

Large pieces of a good grade of white absorbent paper,* 20 by 4½ inches, are soaked in the reagent prepared as follows: 1 Gm. of a good grade of commercial p-dimethylamino benzaldehyde (straw colored) is dissolved in 2 c.c. of concentrated hydrochloric acid and to this are added 98 c.c. of a 2.23 per cent chemically pure oxalic acid. The papers are drained of excess fluid and hung up to dry. They are now stored in the dark in covered glass jars for from five to ten days, during which time most of the hydrochloric acid evaporates. The oxalic acid remaining in the papers is sufficient to give a pH of approximately 2.5 when mixed with body fluids. Once the papers are impregnated, they should not be permitted to come in contact with the skin. The edges of the papers are trimmed off and they are then cut into strips 50 by 8 mm. and stored in suitable lightproof vials. The strips are almost colorless and they will remain without discoloration for over one year. After long standing the test strips become somewhat brittle but this does not reduce their usefulness.

COLOR STANDARDS

A useful adjunct to the test paper is a color chart with standardized color strips. The colors are best reproduced with water colors adjusted to the proper

*A satisfactory paper is Reeve Angel Filter Paper No. 201, 20 by 20 inches, distributed by Reeve-Angel & Co., New York, N. Y.

color by comparison with test strips moistened under the usual conditions with serum containing known amounts of sulfonamide. The standard color strips are mounted on a cardboard of purple hue in order to obtain good contrast. In the absence of a color chart the color comparison may be made with strips wet simultaneously with standard solutions. Standard solutions of sulfonamide in serum keep indefinitely when preserved with merthiolate or metaphen. A single color standard is suitable for the estimation of sulfapyridine, sulfathiazole, and sulfadiazine; for sulfanilamide the values read from this standard should be divided by 1.5. The values for other sulfonamides can be calculated similarly on the basis of molecular equivalence. The color standards are selected in intensities corresponding to 0, 3, 6, 9, 12, 15, and 20 mg. of sulfonamide per 100 c.c. or in other convenient groupings, intermediate values being estimated by interpolation.*

PROCEDURE

Estimation of the level of sulfonamide is made by spreading the solution to be tested evenly over one end of the test strip by means of a fine dropper. Capillary pipettes drawn out from glass tubing are suitable. In this way, the fluid is spread over the paper more by movement of the dropper than by capillarity of the paper fibers, and reagents are not leached out of areas which are oversaturated with the test fluid. Within ten seconds a maximum development of color occurs and the test is evaluated within thirty seconds. To avoid translucence of the paper interfering with the reading, the wet strip is placed on white paper or a porcelain plate. The color is then compared with the graded series of color standards to obtain the sulfonamide value. The color ranges from a pale violet or gray with normal human plasma or serum to a bright orange-yellow with material containing from 15 to 20 mg. sulfonamide per 100 c.c. By a fortunate circumstance, the usual concentrations of sulfonamides attained in the body fall within the range of easily evaluated color intensities. For accurate reading of the colors the operator should be seated comfortably and he should receive evenly diffused light, such as the north light. Facility with the method is attained by practicing with sera of known concentrations for several sessions. Technicians usually become expert in making accurate readings within three or four days. In any event, in the hands of the clinician experienced in the interpretation of sulfonamide levels, plasma or serum concentrations should be readily classifiable into groupings such as "zero," "subtherapeutic," "therapeutic," and "toxic."

Proteins and other substances usually found in normal or pathologic plasma do not interfere with the test, nor do varying concentrations of proteins over a wide range affect the color. In the case of oxalated blood, the test is run on plasma, which is readily obtained by permitting the red cells to settle out on standing or by centrifuging. Serum can also be used and gives the same value. Sulfonamide values for whole blood are not transferable to plasma or serum values, since the latter will be a little higher than the whole blood value. The value given to a standard serum is obtained by a sulfonamide determination run on the corresponding whole blood by the Bratton and Marshall method.

In Table I is shown a comparison of values obtained by the test paper with those secured by the method of Bratton and Marshall. It may be noted that there are very few discrepancies in the values; the maximum deviations are in the higher concentrations and these do not exceed 3 mg., with the great majority

*With the cooperation of the LaMotte Chemical Products Co., Towson, Baltimore, Md., a test kit for this test has been prepared containing color chart, test paper, and capillary tubes.

of the tests within 1 mg. or better. Although the method is subject to the usual errors of visual colorimetry, readings which agree exactly or come within 1 mg. of the Bratton and Marshall method are obtained after some practice. This small discrepancy does not interfere with the accuracy necessary for clinical appraisal of drug therapy, and no false positive values have been encountered.

TABLE I

NUMBER OF BLOOD SAMPLES	BRATTON AND MARSHALL METHOD (MG. %)	LA ROSA TEST PAPER METHOD SHOWING NUMBER OF TESTS DEVIATING BY:			
		<1 MG. %	1 MG. % ±	2 MG. % ±	3 MG. % ±
Sulfadiazine					
	Range				
5	0	5	0	0	0
22	<1	22	0	0	0
56	1-3	51	3	2	0
203	4-8	163	31	9	0
187	9-16	129	43	12	3
14	17-20	5	5	3	1
Sulfathiazole					
2	0	2	0	0	0
4	<1	4	0	0	0
2	1-3	2	0	0	0
20	4-8	13	5	2	0
3	9-13	1	1	1	0
Total 518		397	88	29	4
100%		76.6%	17.0%	5.6%	0.8%

The use of test paper for the sulfonamide determination of blood has a number of advantages over other methods in use at present. Because the volume of plasma required for the test is small, it is not necessary to effect a venipuncture to obtain a sample of blood. Blood drawn from a finger puncture is sufficient for the test. The volume of blood necessary is no more than 0.01 c.c. and this is conveniently collected in a capillary tube 68 mm. long with inside diameter not less than 1 mm.,* containing dried oxalate to prevent coagulation. Nonoxalated capillary tubes may also be used for collecting samples of blood, but sufficient time must be allowed for complete coagulation before proceeding with the test. The oxalated tubes are prepared by filling the capillary tubes with 3 per cent potassium oxalate, followed by drying in a horizontal position in an oven at about 70° C. A large number of these tubes is prepared at one time and, if the diameter is of proper size, no clogging of the tubes is experienced. The capillaries are filled two-thirds full by contact with the drop of blood, and the clear end is then sealed by flaming. The plasma is separated in a centrifuge and the tube is cut at the junction between cells and plasma. The plasma is then transferred to the test paper by holding the open end of the tube perpendicular to the test paper and guiding it down the strip. Transfer of the fluid is easily effected by gravity and the capillary action of the paper. For identification the capillaries are flagged with gummed labels bearing numbers or names. The practicability of this procedure has been demonstrated in our laboratory in a large number of cases. It is especially convenient for use with individuals whose veins are difficult to reach, as well as with babies being treated with sulfonamide. In the case of children, the heel is usually the site chosen for puncture. The method is rapid and reliable as well as economical of equipment and man power, thus making it possible to run several tests on the same in-

*Capillary tubes of these dimensions are supplied by the Kimble Glass Co., Vineland, N. J.

dividual during the course of the day without overtaxing the laboratory personnel. Moreover, a finger puncture is less objectionable and the patient is less disturbed.

The test paper can be used in like manner for the estimation of sulfonamides in other clear body fluids, such as spinal fluid, chest fluid, synovial fluid, and urine. Variation in the amount of protein in the test fluid does not affect the intensity of the colors produced. During chemotherapy the concentration of the sulfonamides in urine is normally too high for a direct quantitative reading, and it is necessary to dilute the urine 1 to 20 to bring the concentration within the range of color evaluation. By confining the test to undiluted urine, important information may be obtained concerning the presence or absence of sulfonamide drug in the body, as in the case of patients who have been transferred recently, or in those receiving prophylactic doses for the prevention of meningitis, scarlet fever, venereal disease, etc.

The reaction on the test paper is given by free sulfonamides and other primary aromatic amines including p-aminobenzoic acid; that is, by the same compounds that give color in the method of Bratton and Marshall. Urea in large concentrations such as are found in urine gives a delayed reaction which is easily distinguishable from a true sulfonamide color reaction. Sulfonamide crystals found in urine are in the conjugated form and when brought into solution do not give a positive test. Pyrrols and indoles do not react with the reagent at the acidity maintained in the test. Moderate hemolysis or pigmentation of body fluids seems not to interfere seriously with color matching. Changes in composition of plasma due to pathologic conditions are not reflected in the color produced and lead to no inaccuracies. Thus the test paper appears to be of general use for the detection of sulfonamides.

SUMMARY

A method is presented for the estimation of the sulfonamide level in blood and other body fluids by the use of test papers impregnated with p-dimethylamino benzaldehyde. The validity of this method has been demonstrated on 518 blood samples by comparing the test paper values with those obtained by the Bratton and Marshall method. The color developed on the test paper is evaluated by comparison with an appropriate color chart or colors developed with sera of known sulfonamide concentration. The method can be used with small volumes of blood drawn in capillary tubes from finger punctures. The test is applicable to plasma, serum, and other clear biologic fluids.

I am indebted to Major Edgar S. Ingraham, Medical Corps, United States Army, Chief of Laboratory Service, for encouragement and cooperation given this work.

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BOOK REVIEWS

Patients Have Families. By *Henry B. Richardson,* M.D., F.A.C.P., Associate Professor of Clinical Medicine, Cornell University Medical College; Attending Physician, New York Hospital; Visiting Physician, Bellevue Hospital, New York, N. Y. The Commonwealth Fund, 1945, New York, N. Y. Price $3.00. Cloth with 408 pages.

This book is a report of a research effort ("The Family Study") supported by the Josiah Macy, Jr. Foundation; the cooperating organizations are Cornell University Medical College-New York Hospital (medicine, psychiatry, public health, medical social service) and Community Service Society (family service, educational nursing). An anthropologist took part during the last months of the two-year study.

During the two years of conferences among the members of the cooperating professions, there developed "a capacity to communicate with each other, to frame questions and to attempt answers," of which they had hardly been aware. It was decided therefore that the report of this work be addressed primarily to the medical profession and to the lay world concerned with medical problems and that it present (a) the value to the medical profession of seeing the individual patient as a personality and as part of his family constellation and (b) the essential contribution which can be made by psychiatry and the social disciplines to this view of the patient and to treatment plans geared to such an emphasis.

The book consists of the experiences which led to the group convictions, an attempt at setting up concepts in terms of which types of family units and family equilibria can be described and uniformities (if such exist) sought for and descriptions of the functions, procedures, and attitudes of the various cooperating professions.

Only about fifteen families were considered at all systematically by the entire group, and these for diverse reasons. Hence it is explicitly stated that the families described are in no way representative of any given area or cultural group.

One of the striking features of the report is the careful description of the history of one family known to various of the professions (chiefly in the hospital). It required two years of intensive medical studies of several of the family members before there appeared in the medical report the slightest hint that their illnesses had any relation to factors not limited to the single organism. The hint was "Inadequate intake of food due to poverty." It required four years before there appeared in the record of one family member a definite statement of a relation between that member's symptoms and "emotional stress." Thereafter, single interviews by psychiatrists and social workers brought out the fact that the supposed "medical" symptoms were temporarily related to difficulties in the interpersonal relations of the family members within the family and outside of it. Treatment could then proceed. Within a statable short period of time the problems of a number of persons could be brought to such a point that the endless waste of medical, social, community and family time, resources, and money

was stopped. Psychiatrists who act as consultants to hospital medical services make such observations regularly, but no one has collected the data systematically and expressed them in terms of such objective criteria as expenditure of time, etc. Until this is done the inefficiency of present-day medicine as compared with its potential effectiveness under the same conditions has not been demonstrated convincingly.

The description of the same family brings out another striking feature of present-day medicine, not stressed by the author of the report. Cornell University Medical College-New York Hospital has had within its walls for some ten years a research group making fundamental contributions to the viewpoint stressed by the author of the report—that of "psychosomatic" medicine. Since 1932 there has also been available a psychiatrist assigned full time by the Payne Whitney Clinic for work as consultant on the medical pavilions. Nevertheless, (about 1940) four years were required by the medical in- and outpatient departments to reach conclusions that demonstrably could be reached in perhaps four interviews, at the most, of one hour each, and were in fact so reached. The inference is warranted that even a first-rate research and teaching arrangement may accomplish little toward the effective teaching of the attitude that the patient must be considered as a behavioral unit and as a member of an interdependent system of such units. Obviously more needs to be known about the elements essential to success. A factual description of the physical setup, organizational relations, and interpersonal relations characterizing the medicine-psychiatry-social work arrangement at any one place such as Cornell Medical College-New York Hospital might be most illuminating.

It is difficult to detect any trace of anthropological influence in that section of the report dealing with the group's attempts to characterize "family equilibrium." The usefulness of the concepts employed is likely to be extremely limited, for they are in no way defined in terms of the operations necessary to establish them. Examples of the concepts are imitation, identification, reciprocating system, focus. Attempts to define in terms of operations the equilibria of pairs of interacting individuals, of families, and of societies have already been made by anthropologists; there is no reference to any of these attempts, some of which have considerable promise.

The book has much of interest in it. It represents a first approach to the scientific study of a most important subject.

GEORGE SASLOW.

Approved Laboratory Technic. Clinical Pathological, Bacteriological, Mycological, Virological, Parasitological, Serological, Biochemical and Histological. By *John A. Kolmer,* M.S., M.D., Dr. P. H., ScD., LL.D., L.H.D., F.A.C.P., Professor of Medicine in the School of Medicine and the School of Dentistry, Temple University; Director of the Research Institute of Cutaneous Medicine; Formerly Professor of Pathology and Bacteriology, Graduate School of Medicine, University of Pennsylvania; and *Fred Boerner,* V.M.D., Associate Professor of Clinical Bacteriology, Graduate School of Medicine and Assistant Professor of Bacteriology, School of Medicine, University of Pennsylvania; Bacteriologist, Graduate Hospital, Philadelphia. Fourth Edition. D. Appleton-Century Company, Inc., New York, N. Y. Price $10.00. Cloth with 1017 pages.

This volume is a veritable encyclopoedia of the most useful clinical laboratory procedures and their normal values. It is intended as a companion volume

to Dr. Kolmer's *Clinical Diagnosis by Laboratory Examinations* and contains, therefore, very little discussion of the significance and interpretation of abnormal results. New sections have been added in this fourth edition on methods for examining saliva, pancreatic function, blood and urine for hormones and vitamins, and stools, blood, and tissues for parasites. Other chapters have been rewritten to include new techniques like the very useful copper sulfate method of Phillips and his co-workers for determining the specific gravity of whole blood and plasma. The material on bacteriologic, mycologic, virologic, and serologic methods is particularly well done. There are valuable unique sections like the ones on the care, inoculation, and bleeding of laboratory animals, recognition of the common diseases of laboratory animals, and the prevention and treatment of laboratory accidents. The book is unusually well printed for a war volume, is profusely illustrated, and contains a wealth of charts. The style is simple, direct, and easy to read.

Hospital laboratories, clinical pathologists, and medical technologists will find the book an essential reference work. Since it must be used in conjunction with the above-mentioned companion volume for clinical purposes, however, the expense involved makes it less serviceable as a textbook for medical students.

The Marihuana Problem in the City of New York. By the *Mayor's Committee on Marihuana.* The Jaques Cattell Press, Lancaster, Pa. Price $2.50. Cloth with 220 pages.